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Interim NPDES (National Pollutant Discharge Elimination System) Compilance Biomonitoring Inspection Manual

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This is one of several monitoring documents produced by EPA to be used in assessing NPDES permit compliance and monitoring the quality of waste water discharges into the Nation's waters.

This particular manual provides guidance to instruct NPDES inspectors in the proper use of those biomonitoring techniques that have been officially recognized for EPA use.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF WATER ENFORCEMENT
COMPLIANCE BRANCH
1979

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DISCLAIMER

This manual has been reviewed by the Office of Water Enforcement, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ACKNOWLEDGEMENT

This manual was developed by a special task force of U.S. EPA and State representatives as a cooperative project in administrative and technical guidance for conducting NPDES (National Pollutant Discharge Elimination System) compliance inspections, and the utilization of biomonitoring techniques. Task force members met in Washington, D.C. and Athens, Georgia and incorporated suggestions and comments from a number of persons representing EPA Headquarters and Regional offices, approved NPDES States, and selected Federal agencies.

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The work group wishes to express its appreciation to all other persons who assisted in the preparation of this manual. Special thanks are due to Mr. Gary R. Polvi, Mr. Brian J. Maas, Ms. Barbara A. Schick, Chief and Environmental Scientists, respectively, of the Technical Evaluation and Support Section, and Mr. George T. Faison of the secretarial staff of the Compliance Branch, Enforcement Division, Office of Water Enforcement. Comments and suggestions for revision and improvements are invited.

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FOREWORD

This is one of several monitoring documents produced by EPA to be used in assessing NPDES permit compliance and monitoring the quality of waste water discharges into the Nation's waters.

This particular manual provides guidance to instruct NPDES inspectors in the proper use of those biomonitoring techniques that have been officially recognized for EPA use.

Other EPA water monitoring publications dealing with NPDES permit compliance are:

- NPDES Compliance Evaluation Inspection Manual, July, 1976.
- NPDES Compliance Sampling Inspection Manual, June, 1977, revised edition, July 1979 - 680 - 197/480.

Also available are the following publications which provide technical information on water monitoring procedures:

- Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents, EPA-670/4-73-001; July, 1973.
- Basic Water Monitoring Program, EPA-440/9-76-025, 1977; Revised Edition, May, 1978.
- Model State Water Monitoring Program, EPA-440/9-74-002, June, 1974.
- Bioassay Procedures for the Ocean Disposal Permit Program. EPA 600/9-78-010; Revised Edition, March 1978.

The NPDES compliance inspection program represents a significant commitment of resources by the States and EPA to verify permit effluent limitations and to assure that compliance with permit requirements for monitoring, reporting and compliance schedules are being met.

Biomonitoring conducted in a thorough, professional manner in accordance with procedures described in this Manual, provides the EPA, States, and permittees with a practical tool for checking on the effectiveness of compliance with NPDES permits and the overall National Enforcement program. Familiarity with this Manual by EPA inspectors is essential; its use by other interested and concerned persons (States, permittees, consultants, etc.) is encouraged.

Because EPA policy and specific biomonitoring requirements and techniques have not been finalized, this manual is being published as interim guidance. However, it is imperative that plans and methods for biomonitoring be initiated now so that problem areas can be discovered and addressed before final policy is implemented.

Readers are encouraged to offer comments or suggest revisions. These should be directed to the NPDES Compliance Biomonitoring Manual Review Committee, Technical Evaluation and Support Section, Compliance Branch (EN-338), Enforcement Division, Office of Water Enforcement, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, D.C. 20460.

NPDES COMPLIANCE
BIOMONITORING INSPECTION MANUAL

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SECTION I

INTRODUCTION

A. Background

The Federal Water Pollution Control Act Amendments of 1972 established the objective of restoring and maintaining the chemical, physical, and biological integrity of the Nation's waters. This objective has remained unchanged in the 1977 amendments to the Act, commonly referred to as the Clean Water Act of 1977. To achieve this end, the Act sets forth a series of goals, including the goal of eliminating the discharge of pollutants into navigable waters by 1985. The principal mechanism for reducing the discharge of pollutants is through implementation of the National Pollutant Discharge Elimination System (NPDES) established by Section 402 of the Act.

NPDES permits have been issued to approximately 55,000 municipal and industrial point sources. Permits contain four primary elements: (1) final effluent limitations reflecting statutorily required treatment levels; (2) interim effluent limitations until the attainment of final effluent limitations; (3) construction schedules for the achievement of final effluent limitations; and (4) reporting requirements relating to compliance with effluent limitations established for each parameter limited in the permit for both interim or final effluent limitations and compliance with construction schedules.

Compliance with these effluent limitations and self-monitoring requirements of NPDES permits is assessed by the regulatory agency through a combined program of self-monitoring data review and facility inspections.

Historically, the major focus of final effluent limits contained in NPDES permits has been the control of conventional pollutants such as BOD, pH, fecal coliform, etc. At the time of first round permitting, these pollutants were considered the parameters which most urgently needed controls.

In second round permitting, however, Agency emphasis is shifting from the conventional pollutants to the control of toxic pollutants. This is due in part to the NRDC Consent Decree which identified 65 families of toxic pollutants for which both ambient and effluent guidelines must be developed. Furthermore, this shift is the next logical step in water pollution control as the potential for environmental harm from conventional pollutants decreases due to compliance with effluent and water quality standards.

Traditionally, NPDES permits have contained specific numerical limits for individual chemicals. Similarly, effluent guidelines which are established for Consent Decree toxic pollutants under Section 307 (a), will also be chemical specific numerical limits. However, this approach has several drawbacks. It does not take into account synergistic toxicity resulting from a combination of two or more chemicals in an effluent, nor can it be expected to always provide an adequate margin of safety for chemicals which lack specific toxicity data. In addition, periodic sampling and analysis for these compounds during NPDES self-monitoring activities is extremely resource intensive and many times the methods for analysis are not completely reliable.

In general, since the effluent guidelines will be technology based, they will not always be able to guarantee water quality sufficient for the protection of indigenous aquatic life. Because of this, the Act provides for methods of developing more stringent water pollution controls, such as water quality standards which are based on specific biological toxicity data. It is because of these issues and Section 308, which specifically provides for biological monitoring, that the NPDES Biomonitoring Program has been developed. Since biomonitoring is a measure of the direct acute toxicity of the effluent, it provides a good estimate of the effluent's potential for environmental harm and its potential effect on indigenous biological communities. In addition, biomonitoring provides a method for assessing the adequacy of and assuring compliance with State water quality standards.

Simply stated the objectives of the biomonitoring program are as follows.

1) To serve as a screening mechanism, isolating toxic conditions in effluents which may not have been detected through routine chemical analyses;

2) To evaluate effluents that are in compliance with State water qualtiy standards;

3) To provide a monitoring function which will act as an early warning system for toxics which may or may not be controlled through BCT/BAT and;

4) To serve as a surrogate test for toxic conditions as a prerequisite and priority setting process for the more resource intensive chemical analysis for toxic pollutants.

B. Purpose of Manual

The NPDES Biomonitoring Inspection Manual is primarily designed to aid in the conduct of NPDES compliance inspections by EPA and State agency personnel and will also be of use in the orientation and training of any technical personnel. Definitions of selected terms used in this manual are presented in Appendix H.

This manual is intended to assist EPA in meeting its statutory obligations under section 308 and other pertinent parts of the Clean Water Act, of 1977 (see also section I C. of this manual). In addition, this manual will be of assistance in fulfilling the following Agency needs:

1. To provide Agency-wide policy and a uniform procedural, administrative, legal and technical framework which all of EPA's Regional Surveillance and Analysis (S&A) personnel and State agency employees can use as guidance. This uniform reference is an important step in making biomonitoring data and administrative information more valid and of greater use to enforcement offices. Furthermore, data obtained in this manner can greatly contribute to the continued refinement and development of biomonitoring techniques.

2. To simplify NPDES biomonitoring activities and standardize techniques for performing toxicity tests.

3. To provide administrative and procedural support to the Permits Biomonitoring Policy Guidance manual for the NPDES Permits Program which establishes guidelines for the inclusion of biomonitoring requirements in NPDES permits.

For the purpose of addressing the technical aspects of aquatic acute toxicity testing this manual has incorporated in its entirety the EPA report: "Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms", EPA-600/4-78-012. Revised July 1978.

C. Statutory Authority

Biological monitoring (biomonitoring) is the logical and appropriate method for ascertaining the "...biological integrity of the Nation's waters." No other method is likely to accomplish the monitoring needed to check on the desired restoration and maintenance of biological integrity.

Section 308(a) of the Act states in part:

> (A) the Administrator shall require the owner or operator of any point source to (i) establish and maintain such records, (ii) make such reports, (iii) install, use and maintain such monitoring equipment or methods (including where appropriate, biological monitoring methods), (iv) sample such effluents (in accordance with such methods, at such locations, at such intervals, and in such manner as the Administrator shall prescribe), and (v) provide such other information as he may reasonably require; and
> (B) the Administrator or his authorized representative, upon presentation of his credentials --
>> (i) shall have a right of entry to, upon or through any premises in which an effluent source is located or in which any records required to be maintained under clause (A) of this subsection are located, and
>> (ii) may at reasonable times have access to and copy any records, inspect any monitoring equipment or method required under clause (A), and sample any effluents which the owner or operator of such source is required to sample under such clause.

The Administrator is specifically authorized by the provisions of section 308 to install, use, and maintain monitoring equipment and methods, including biological monitoring, to determine "...whether any person is in violation of any effluent limitation, or other limitation, prohibition or effluent standard, pretreatment standard, or standard of performance..."

Thus, the use of biomonitoring techniques for NPDES compliance is clearly recognized and authorized by statute.

D. Biomonitoring Requirements in Permits

At the option of the permitting agency, NPDES permits may contain specific enforceable toxicty limits or biological self-monitoring requirements. In many cases, permitting authorities may elect to include biological self-monitoring requirements without toxicity limitations, such as when data on the potential toxicity of the effluent has not been well documented. While data obtained during biological self-monitoring from facilities which do not have specific permit toxicity limitations may not be directly used as the basis for enforcement, it can be used 1) in enforcement case development, 2) to identify eminent health hazards, 3) to flag possible permit violations of chemical parameters and/or identify potential toxic conditions in the receiving waters. For these reasons enforcement offices may also elect to perform biomonitoring inspections at any time regardless of whether there are biomonitoring requirements in a NPDES permit.

E. Federal and State Cooperation

Congress has directed that the EPA seek, encourage, and support cooperation with the States (Sections 101, 102 and 103 of the Clean Water Act of 1977).

EPA personnel involved in planning and conducting biomonitoring inspections for NPDES compliance should at all times, be aware of the value and need of coordinating Federal inspections and other activities and programs with the appropriate State officials and agencies. This should be a reciprocal arrangement, with State Agencies also coordinating inspections with EPA. This essential coordination depends on a continuing commitment to inform the appropriate EPA State representatives of plans and intended activities concerning NPDES compliance monitoring in a particular State.

Cooperation between EPA and the States must be promoted on a continuing basis to be successful, and if done will reap the benefits of more effective control and management of the NPDES program.

SECTION II

LEGAL CONSIDERATIONS

A. Access and Warrants - Constitutional and Statutory Requirements

Inspectors must gain entry to a permittee's private premises and perform all subsequent inspection activities in a manner consistent with the right of privacy of the permittee as guaranteed by the U.S. Constitution. Legal entanglements may result if entry or access to any type of evidence is obtained through direct or implied threats, or trickery. Any information obtained in this fashion may not be admissible as direct evidence in administrative or court enforcement proceedings.

The "unreasonable search and seizure" requirements of the U.S. Constitution apply to the activities of all government employees. These requirements mean that entry into a permittee's private property for inspection purposes must be done only with the consent of a person authorized to give it, or by the authority of an administrative or criminal search warrant issued by a judge or magistrate, having jurisdiction.

In almost every case, EPA and State personnel will attempt to obtain entry and perform their inspection or monitoring responsibilities on the basis of consent of the owner, operator or person in charge of the facility, that is, a person who has authority to give consent. In the case of announced inspections, this can be the person the inspector has been directed to ask for, even if they are not the plant manager.

Federal inspectors must also comply with the requirements of section 308 of the Clean Water Act. That section requires Federal inspectors to present their credentials at the time they seek access to a permittee's premises.

Appendix J of this manual includes copies of memoranda from EPA's Assistant Administrator for Enforcement which provide guidance in the conduct of inspections in light of the recent Supreme Court decision in Marshall v. Barlow's Inc.

Both the constitutional and statutory requirements apply to individuals and corporations.

B. Discussions with Permittees or Their Agents - Privilege Against Self-Incrimination

During the course of an enforcement effort, sufficient information may become available to have reason to believe that a particular person has violated a Federal law. A likely possibility is the determination that a particular person may have knowingly made a "false statement.....in [a]...document filed or required to be maintained under [the Clean Water Act]", such as in a logbook or report to the permitting agency; or has falsified, tampered with, or rendered inaccurate a monitoring device or method required to be maintained under the Act, such as a biomonitoring device or an ancillary measuring device. Such declarations or actions constitute Federal crimes under section 309(c) of the Act. Persons (but not a corporation) who have violated section 309(c) may not be required to testify against themselves. In addition, any enforcement leads obtained from information developed from statements made by suspects who have not given notice of and voluntarily given up their Constitutional right to remain silent may likewise not be available as evidence against these persons in a criminal trial.

Further, section 308 of the Act does not give inspectors authority to do anything other than enter, inspect and copy records, inspect monitoring equipment and sample effluents. The inspectors must limit their discussions with permittees' personnel to requesting basic information required to enable the inspectors to perform their duties. This admonition applies to State inspectors, if State law includes State offenses equivalent to those in section 309 of the Act.

C. Expert and other Testimony

Facts and opinions derived from inspections often constitute testimony before administrative bodies and courts. Not everything that is seen or heard, however, may be admitted. The two exceptions which may lead to the exclusion of testimony of inspectors are relevance and hearsay. Any information which will not aid the finder of fact to determine an issue in a case is not relevant, and is therefore inadmissible. Thus, the results obtained from the bioassay of samples taken at a point other than the specified sampling point referred in an NPDES permit would probably be held by a judge to be insufficiently related to a determination of toxicity at the designated point, and would therefore not be allowed in evidence. Similarly, with few exceptions, direct testimony may only be given by a witness on facts based on personal knowledge. Thus, in most situations, a statement like "I know it's true because Henry told me so" constitutes hearsay and will not be admissible to prove the truth of whatever Henry said.

In testifying, inspectors who may be acting as ordinary witnesses, are authorized not only to state facts known to them, but may also testify as to their opinions in their area of expertise. Technical matters, such as the propriety of sampling or an opinion as to the results obtained from a particular biomonitoring experiment are examples of expert opinions which are admissible evidence. In order to qualify as experts, persons must show that their opinions on scientific matters will assist the trier of fact, and that they are qualified as experts in that field by virtue of their knowledge, skill, experience, training or education.

D. Chain of Custody and Preservation of Documents

Results obtained by persons having expertise in conducting sampling and biomonitoring (see Subsection 3, above) are, in fact, valid. However, an additional effort in custodial care is absolutely required to assure the admission of that information in enforcement proceedings. A recent paper authored by expert EPA biologists described this issue as follows:

> The only difference between normal scientific prudence and legalistic practice is an added stress upon written accountability.*

This "written accountability" requires the maintenance of chain of custody records, detailed recording of all aspects of sampling and biomonitoring, and the preservation of that documentation.

In the context of biomonitoring done on-site, chain of custody records are those which show the source of the materials tested and which indicate that test results are not rendered inaccurate by deliberate tampering or unintentional error.

In terms of biomonitoring done off-site, chain of custody consist of records and/or labels, field data sheet and/or field log book showing where, when and by whom a sample was taken and the persons to whom custody was relinquished throughout the sampling and testing process, together with appropriate notations in the laboratory log book listing the names of sample custodians as well as notations on the security measures taken to protect the integrity of the sample during testing. Appendix E contains samples of a chain of custody record and sample labels similar to those used in one of EPA's laboratories but modified for specific use with bioassay samples.

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*Prager, Flemer and Browne. "Some U.S. Concerns in Obtaining and Using Non-human Tissue Samples," 1979

Both on-site and off-site testing require that appropriate chain of custody be maintained. Off-site testing, of course, will also include intermediate transportation information. Log books must contain all pertinent information.

The "written accountability", as it relates to the actual biomonitoring, consists of detailed notes of all the inspector's actions and observations related to the biomonitoring testing. Furthermore, all notes taken during the course of biomonitoring tests must reflect total compliance with EPA official protocols and procedures, which include acclimation of organisms, quality assurance and chain of custody. Notes must also show unusual occurrences which may bear on the propriety or impropriety of the results. All notes must be made as close in time as possible to the event that they describe. Notes must be complete and coherent if they are to show that the inspector's opinions are worthy of consideration. Materials and observations which in fact are considered relevant, even if they do not fit into a "normal" pattern, must not be skipped or deleted. Only if this is done can there be documented assurance of the inspector's objectivity.

All chain of custody documentation, including log books, etc. should be retained for no less than three years after testing has been completed. Any unused portions of samples should also be retained until the enforcement office authorizes disposal. The transfer of test data and/or results to permanent non-bulky files such as microfiche could be of great use in the study of environmental quality trends and in testing protocol evolution and improvement. When results and samples are needed, as in cases of active litigation, they shall be retained until case resolution. Although documentation is performed by the analyst, the documents themselves are the property of the employee's agency. The inspector should turn over all such documentation, in accordance with agency procedures, to the person or persons who are the permanent custodians of such documents. When these documents are held in custody in this manner, they are retrievable when needed. As an example, Appendix G contains the document handling procedures utilized by EPA's National Enforcement Investigations Center (NEIC).*

E. Relations with the Public

1. Financial Activities and Conflicts of Interest

Inspectors must conduct their business affairs in a way that there can be no suspicion that their activities are motivated by expectations of personal gain. They generally should not make investments in or accept outside employment or gratuities from persons whose activities they regulate. Aside from exercising personal discretion in these matters, inspectors routinely should review their agency's related instructions. EPA's instructions are found in Title 40, Chapter 3 of the Code of Federal Regulations, and published as "Responsibilities and Conduct of EPA Employees".

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*NEIC Policies and Procedure Manual, 1978. National Enforcement Investigations Center, Denver, Colorado.

2. Appearance and Dress

Inspectors recognize that to the public they are the embodiment of their agency and that they must represent it in the best possible light. Inspectors must therefore appear neat and clean while performing their duties. This does not mean, of course, that inspectors should not dress in a manner appropriate to protect themselves from climatic conditions or from job-related hazards (see Appendix F).

3. Communications with Permittees and other Members of the Public

It is important that cooperation be obtained and good relations established when working with the permittees and the public. This can best be accomplished by politeness, diplomacy and tact. Everyone, even hostile persons, should be treated with courtesy and respect.

During conversations with permittees and/or their representatives, the inspectors should refrain from speculating over inspection findings or asking any questions beyond those actually needed for inspection purposes. Inspector behavior and conversation should reveal know-how and objectivity.

4. Disclosure of Official Information

Inspection personnel must always refrain from answering any questions or making any statements which might endanger any of the permittee's proprietary materials, right of privacy, or which may in any way interfere with a monitoring or enforcement activity. Thus, an inspector should not discuss any matters related to a permittee whose effluent is being monitored without having first obtained approval from the appropriate EPA or State enforcement official.

SECTION III

PLANNING BIOMONITORING INSPECTIONS

A. Pre-inspection Planning Activities

Pre-inspection planning will insure the best and most efficient use of resources. Pre-inspection planning activities can be divided into the following categories:

1. Selection Coordination

Coordination in the selection of candidates for inspections should involve the exchange of information with other related programs. Offices with enforcement authority, however, will make the final selection of candidates for inspection. Factors useful in selecting candidates for inspections are contained in Appendix I. These can be used by inspectors and other personnel involved in NPDES inspections to assist them in their advisory role to enforcement efforts. Written notification to the permittee is usually conveyed by means of a 308 letter. Letters of the 308 type perform three basic functions: 1) they inform the permittee of the upcoming inspection usually without specifying a date, 2) they detail statutory authority to inspect, and 3) they address safety and information matters associated with actual inspection.

Each EPA Regional office can continue to use its present 308 letter or other method of notification or it may want to introduce alterations that would specifically accommodate biomonitoring inspections.

2. Selecting the Type of Biomonitoring Inspection to be Conducted

If not specified by the enforcement program, the inspection team may also have to decide on the type of biomonitoring inspection it will conduct on a particular permittee (see flowchart on page I-6). Flow-through bioassay inspections are very resource intensive and their use as a routine inspection tool is not advisable. The office with enforcement authority may find it advantageous to develop and use specific guidelines for determining the type of biomonitoring inspection to be employed. Various types of biomonitoring inspections are described later in this section.

3. Some Basic Information, Personnel and Material Needs

All biomonitoring teams must include, as one of its members, at least one inspector experienced in biomonitoring techniques. The only exception to this rule is the collection of a composite or grab sample for off-site static bioassay testing. In this case, any member of the inspecting team may be supplied with sample tags, chain of custody records, adequate sample containers and careful instructions on sampling procedures. These instructions may be found in the NPDES Compliance Sampling Inspection Manual. The type of sample to be collected (grab, composite) will be specified by either the permit conditions, if appropriate, or an experienced biologist. The experienced biologist will also determine the volume and preservation techniques to be employed.

Minor equipment items and supplies should be duplicated to avoid unnecessary delays in case of failure or breakage. The EPA bioassay technical manual (see Appendix A) is the source from which to prepare a materials and supplies checklist. The field personnel should make it a habit to go over a checklist of supplies and equipment prior to each departure. The checklist should include, among other items, the following:

a. A copy of the latest NPDES and applicable State permits.

b. A sketch or copy of a U.S. Geological Survey map showing the facility location and/or its effluent discharge points (Overflow and bypass discharge points should be identified).

c. A summary of names, titles, locations and phone numbers of the responsible persons (operators, municipal or industrial officials) involved with the permittee's waste water control program.

d. A flow chart or summary of the present and/or planned treatment facilities. (If appropriate, include industrial production processes.)

e. Information from or copies of previous inspections and self-biomonitoring reports (including quality assurance data).

f. Information from the permittee's most recent Compliance Schedule Report and/or Discharge Monitoring Report (DMR).

g. Letter of notification of inspection to the permittee and the response (if applicable).

h. Information from any other recent correspondence and/or regulatory action, noting the status of requested actions and/or compliance with enforcement actions.

i. Previous EPA biomonitoring studies, consultant's reports and laboratory test procedures.

j. Evaluation form for rating self-monitoring practices associated with biomonitoring.

k. The NPDES Biomonitoring Inspection Manual.

Reports and data from the above checklist are used in evaluating the permittee's compliance record with NPDES permit requirements.

Knowledge of the distance and location of the sampling point, electrical power supply and dilution water sources should be used when positioning the mobile laboratory.

If the inspector foresees the need to transport or ship samples of any sort, he should obtain in advance the necessary containers and agency labels. Proper chain of custody procedures should be followed (see this manual, page II-5). For the proper transportation procedures, see the U.S. Department of Transportation (DOT) regulations and EPA/DOT agreements.

An important part of the pre-inspection activities includes obtaining information on plant regulations such as gatehouse check-in and security procedures, in-plant travel restrictions and safety regulations. Good planning includes providing the inspection team with proper equipment and attire to meet all safety regulations.

B. Coordination of Inspection Activities

1. Coordination with the Permittee

Prior to contacting the selected permittees, members of the the inspection team have already conducted a comprehensive review of the permittee's compliance record.

Once the nature of the permittee's effluent has been studied, and their compliance history and present compliance status determined, the inspection team leader may want to establish direct contact with the permittees to give them an approximate inspection date and check on the availability of special equipment such as necessary power supply. If a 308 letter has been sent and the permittee has responded, this call may not be necessary. If there is direct verbal communication with permittees, such details as plant operations, production levels, and related information can be obtained. Further, direct exchange of information between the permittee's representatives and the inspecting team may facilitate the actual inspection process and prevent misunderstandings from occurring. The following are some important items of information that can be obtained from direct verbal contact with permittees:

a. Name, telephone number, mailing address and work schedule (for immediate period when inspection might occur) of the person that the permittee has designated as the contact for inspection purposes.

b. Plant schedule and operational levels.

c. Permittee's in-plant safety requirements and availability of necessary safety equipment.

d. Accessibility to adequate source of dilution water.

e. Location and ease of acccess to sampling points.

f. Availability of electrical power supply.

The inspection team leader may want to inform the permittee of some or all of the following information:

a. Inspection Time Frame - (Approximate Inspection date). For example, inspection will take place within the next sixty days.

b. Purpose of Inspection - Is the purpose to check NPDES permit conditions to assess quality assurance of self-monitoring data, or to establish effluent limits?

c. Nature of the Inspection - What kind of biomonitoring inspection will be conducted? Will there be any other type of analyses associated with the survey? (i.e. effluent chemical samples, check samples, laboratory evaluation).

d. Scope of the Inspection - Number and location of sites to be sampled, amount of samples to be taken, etc.

e. Who is Going to Participate? - How many people are going to be involved in the survey and, if possible, who are these persons? Is any other agency going to be involved? What part, if any, will the permittee be expected to carry out? Will the company want to duplicate any portion of the survey or receive split samples for chemical analyses?

2. Coordination with Other EPA Programs

There are cases in which the biomonitoring inspection of a permittee will be conducted simultaneously with a normal Compliance Sampling Inspection (CSI). This type of combined inspection must be carefully coordinated to maximize utilization of traveling and manpower resources. In this case the biomonitoring inspection team should collect and prepare samples for chemical analysis of the effluent on which the bioassay is being performed. In this case advance scheduling of analytical services should be coordinated with the receiving laboratory.

3. Coordination with State and Other Agencies

Valuable information may be obtained from State and other government agencies. This coordination should take place as far in advance as possible. Information obtained from these agencies should corroborate information obtained from the permittee.

The following is some of the information that may be obtained from other government agencies.

a. Copy of the latest NPDES permit.

b. Permittee's self-monitoring data.

c. Ambient stream data upstream and downstream from facility.

d. Results and reports of recent compliance inspections.

e. Reports of fish kills or spills in the stream in question attributed to the permittee.

f. Any knowledge of operational problems or changes in plant processes.

g. Recent correspondence and telephone communications with the permittee.

h. Is the State or other agencies interested in participating in the survey?

SECTION IV

INSPECTION TYPES

A. Announced and Unannounced Inspections

Both announced and unannounced inspections require essentially the same planning and preparation except for the pre-inspection facility reconnaissance. Unannounced inspections will probably require schedule adjustments for this purpose.

For example, delays may occur at the start of unannounced inspections as permittee personnel are diverted from their normal duties to accommodate the unexpected inspection activities. However, if a 308 letter has been sent previously, this delay should be minimized. The same information that is supplied to permittees in advance for announced inspections will have to be supplied quickly and concisely at the beginning of unannounced inspections.

Although it is reasonable for the permittee to cause delays during unannounced inspections, unreasonably lengthy ones should not be permitted without adequate justification.

B. Sampling Type Inspections

A biomonitoring sampling inspection is an evaluation of an effluent based on actual sampling and testing. This is referred to as a sampling-type inspection so as not to be confused with a Compliance Sampling Inspection (CSI) which involves the taking of samples for subsequent chemical analysis. The inspection consists of collecting an effluent sample and determining its biological impact by exposing test organisms to the sample. Biomonitoring tests are performed according to specifications in the NPDES permit and the published EPA test protocol (see Appendix A).

1. On-site Biomonitoring

On-site biomonitoring inspections of a permittee's effluents includes a combination of the following depending on the specific needs of the inspection:

- An 8 to 24 hour range finding static bioassay (usually allowed to run for 24 hours).
- A 24 hour static bioassay.
- A 96 hour flow-through bioassay.
- A 24 hour QA bioassay with reconstituted water and a standard toxicant.
- Careful examination of the permittee's records and laboratory facilities (see flowchart page I-6). If a permittee has a biomonitoring requirement, the type of biomonitoring test performed during the inspection should be the same as the test required by the permittee's NPDES permit.

a) 96-Hour Flow-Through Bioassay

This is one of the most complete of all on-site sampling type biomonitoring inspections. The methods and materials to be utilized in conducting this type of biomonitoring inspection are fully described in Appendix A and Section V (Quality Assurance) of this manual.

b) 24-Hour Static Bioassay

The 24-hour static bioassay testing of an effluent grab sample may be enough in those cases where permittees can prove that proper mixing and/or long retention time produces a homogenous effluent. In other cases, a static bioassay using a 24-hour composite sample or a 96-hour flow-through bioassay must be conducted. For further details on sampling and holding time see pages 17 and 18 of Appendix A.

2. Off-site Testing

This type of sampling inspection consists of collecting a sample of the permittee's effluent and transporting it to EPA or State laboratories to be tested.. The sample may be a grab sample or a 24-hour composite sample.

A 24-hour static bioassay would usually be the preferred off-site test.

C. Evaluation Inspections

1. The Compliance Evaluation Inspection (CEI)

This is the simplest and less resource intensive type of the evaluation inspections. It consists of an examination of the permittee's records, laboratory and production facilities. As with all other types of inspections, the inspector, prior to the visit, must become acquainted with the permittee's compliance track record and all relevant aspects of the production and waste treatment operations.

No inspection team is necessary for this type of inspection. An experienced inspector with knowledge in biomonitoring suffices.

Because of its simplicity and few pre-inspection needs, this is the type of evaluation inspection most likely to be conducted effectively on an unannounced basis. Refer to the NPDES Compliance Evaluation Inspection (CEI) Manual (EPA, July 1976) and to Appendix C-1 of this document for additional guidance.

For purposes of satisfying EPA inspection program resource commitments, a CEI shall have the same classification or resource accounting code irrespective of whether it relates to biological, chemical or combined evaluation of permittee records and facilities.

2. Performance Audit Inspections (PAI)

A biomonitoring performance audit inspection is an announced, non-sampling audit of the permittee's records, monitoring and analysis procedures, which assesses the accuracy and completeness of all aspects of biological monitoring and reporting requirements of NPDES permits. The performance audit inspection (hereafter referred to as an audit) serves as a compromise between a simple compliance evaluation inspection and a comprehensive sampling inspection. It eliminates the short-comings of a CEI which does not include actual observation of the permittee's self-biomonitoring procedures. Furthermore, audits are not as resource intensive as sampling inspections which can continue for several days or weeks with round-the-clock sampling of numerous wastewater outfalls.

Because of its manpower efficiency, the audit is a very useful tool in any compliance monitoring program. Not only does it require a minimum number of personnel, but it eliminates extensive on-site sampling and analyses while permitting in-depth observation of the permittee's biomonitoring procedures. It functions as an on-site overall quality control examination. An audit is a highly efficient mechanism for detecting self-monitoring or permit limitation deficiencies. Therefore, results of an audit can be the basis for planning corrective measures and/or initiating comprehensive sampling inspections by specialists to obtain details of identified problems.

For purposes of satisfying EPA inspection program resource commitments, an audit (PAI) shall have the same classification or resource accounting code irrespective of whether it relates to a biological, chemical or combined audit of the permittee's self-monitoring program. The following are some of the most important components of an audit type inspection:

a. Direct observation and audit of the permittee's bioassay procedures during the course of a complete self-biomonitoring test.

b. Examination and audit of the permittees:

    1) Records (includes DMRs and self-monitoring)
    2) Chain of custody procedures
    3) Data collection and evaluation methodology
    4) All laboratory facilities used in performing biomonitoring tests.

SECTION V

QUALITY ASSURANCE

Quality Assurance (QA) practices for effluent bioassays include all areas that affect the accuracy and precision of the data, such as: effluent sampling and handling; the test organisms; equipment; test conditions; instrument calibration; replication; reference toxicants; record keeping; data evaluation; and chain of custody. Some of these areas are addressed in the following discussion.

A. Effluent Sampling and Handling

Continuous effluent sampling and flow-through bioassays are the most sensitive and reliable testing methods. If continuous effluent sampling is not possible, flow-proportional composite samples, composite samples or grab samples may be taken. If samples are collected for offsite testing, the sample preservation procedures given in Appendix A, page 18 should be observed. Any samples that are to be tested two or more hours after collection should be kept chilled at 4°C (use only frozen water ice). No air space should be left between the sample's surface and the container's cap. Even when sample preservation procedures are properly observed, testing should always begin within 24 hours after collection. Toxicity data from effluent samples that have been held more than 24 hours prior to testing may not be used unless it can be proved that the toxicity of the sample has not changed because of excessive holding time.

In running a static test, the volume of sample needed will depend on the number of dilutions to be tested, test-temperature and the test organisms loading factor. A five (5) gallon sample may be enough for Daphnia or other test organism of similar size. For larger test organisms such as the fathead minnow, volumes up to 15 gallons may be needed.

B. Test Organisms

For the 24-hour bioassay screening test, with receiving waters having a salinity less than five 0/00 (parts per thousand), it is recommended that the fathead minnow (Pimephales promelas) be used. Test results from 24-hour static bioassays using juvenile instead of adult fathead minnows have been found to correlate more closely to test results from 96-hour flow-through bioassays. For this reason it is recommended that the fathead minnows used should not be more than 3 months old nor less than 2 weeks.

With receiving waters having a salinity of greater than 5 0/00, the mysid shrimp (Mysidopsis bania) is recommended.

Permittees or NPDES inspectors may elect to run additional tests with other species at any time. These may include species indigenous to the particular receiving waters.

The fathead minnow and mysid shrimp are recommended rather than a sensitive indigenous species for the following reasons:

1. This gives a simplified national approach where toxicity data from any NPDES permittee can readily be correlated with all others.

2. Data does not always exist as to which species is the most sensitive indigenous species.

3. Many times, because of an effluent's impact on a receiving water, all sensitive resident species have previously been eliminated and only extremely pollution tolerant species remain. Using these would give an unrealistic estimate of the effluent's effect on the receiving waters.

4. Often, the sensitive indigenous species are not commercially available in all areas and would represent significant costs to the permittee.

5. Both the fathead minnow and mysid shrimp are easily raised under laboratory conditions and permittees and commercial labs would be able to maintain their own stocks.

Test organisms should not have been exposed to pollutants or other stress prior to biomonitoring, and should be free of disease. Holding conditions should conform to Agency recommended procedures (see Appendix A).

C. Facilities and Equipment

Laboratory and bioassay temperature control equipment must be adequate to maintain recommended test water temperatures. To ensure electrical power, an independent, portable generating unit is recommended. Thermometers, pH meters, dilutors, and other measuring devices must be calibrated by the manufacturer's recommended methods before and at appropriate intervals during use. Glass, stainless steel and perfluorocarbons are to be used for test chambers, tubing, etc. (see Appendix A). All equipment must be carefully cleaned.

D. Dilution Water

Receiving water should be used as dilution water wherever possible. However, if the receiving water has been influenced by other point-source discharges, contains toxic substances or is otherwise unsuitable as dilution water, a chemically equivalent or "reconstituted" water may be employed. (see Appendix A, p. 14.) This water must have a total hardness, total alkilinity and specific conductance within 25 percent, and pH within 0.2 units of the receiving water at the time of testing.

E. Test Conditions

Water temperature ranges for the test organisms must be maintained within the specified limits. Dissolved oxygen concentrations should not fall below 60 percent saturation for cold-water species and 40 percent saturation for warm-water species. Loading limits of organisms in test chambers must not be exceeded. Duplicate test chambers should be provided at each concentration of effluent (for further details see Appendix A).

F. Reference Toxicants

Reference toxicants are used to establish the validity of effluent toxicity data generated by bioassay laboratories. Factors affecting the accuracy of the data include test organism condition and sensitivity, water temperature, and dilutor calibration. The condition and sensitivity of each batch of test organisms to the reference toxicant should be determined at least once each month. No batch of test organisms should be used in an effluent bioassay unless its condition has been checked against the reference toxicant within the preceding 7 day period. If preferred, this sensitivity test may be run on-site in the mobile laboratory concurrently with the effluent bioassay.

In programs conducting more than one sampling inspection per month, it may be convenient to run all organism sensitivity tests on-site, concurrently with the effluent bioassays. The reference toxicant, sodium dodecyl sulfate (SDS), should be used in a 24-hour static bioassay, with moderately-hard (Hardness = 160-180 mg/l) reconstituted water. A minimum of three reference toxicant concentrations should be employed: one above, one equal to, and one below the LC50.

The 24-hr LC50 of SDS for fathead minnows (Pimephales promelas) of 2 weeks to 3 months of age should fall in the range of 2-7 ppm. The 24-hr LC50 of SDS for Mysidopsis bahia should fall in the range of 2-8 ppm.

If the LC50 of SDS does not fall in the recommended range for the test organisms, the sensitivity of the organisms and/or the overall credibility of the test system are suspect. In this case the test system should be examined for defects. A different batch of test organisms should be employed in the effluent toxicity test.

G. Record Keeping

Proper record keeping is very important. Bound notebooks should be used to maintain detailed records of test organisms, and equipment such as: supply source, date of receipt, culture maintenance, disease treatment, calibration of the dilutor and other equipment, and test conditions.

Annotations should be made as close to real-time as possible to prevent the loss of information.

SECTION VI

HEALTH & SAFETY

A. General

The same safety rules that are in effect in a fixed laboratory will apply to all mobile laboratory operations. Evaluation and sampling inspections at wastewater treatment facilities may involve significant risks to personal safety and health. Personnel should protect themselves from injury which may result from unsafe conditions or practices at the inspection site by taking all safety precautions necessary for the:

1. Prevention of bodily injury, and contact with corrosive and/or toxic substances.
2. Prevention of infections.
3. Prevention of asphyxiation due to lack of oxygen or presence of noxious gases.

Prior to site visit, inspection personnel should make sure that all necessary safety equipment and materials have been obtained and are in good condition.

B. Personal Conduct

Every work assignment must include review and evaluation of all potential hazards involved. No job shall be started until the worker is convinced that all safety factors have been considered. Care should be taken not to impede or disrupt normal performance of industrial employees at work site.

C. Safety Equipment

1. Personal Safety Gear

While on duty, inspection personnel should use the safety equipment as required:

2. Laboratory Safety Equipment

Each lab (including mobile labs) should have safety equipment such as first aid kit, fire extinguisher, fire blanket, etc.

Inspectors should contact the permittee and inquire as to any special safety measures and equipment requirements that must be observed at the permittee's installation. This precaution may save the inspectors from unnecessary problems and delays and provide protection from injury.

D. General Laboratory Operation

1. Work with hazardous materials must be performed in compliance with special rules pertaining to such materials. Hazardous work will not be performed alone in a mobile laboratory or anywhere else.

2. All containers will be adequately labeled to indicate their contents.

3. Good housekeeping contributes to safety and credibility.

4. The use of electrical equipment or cords not having Underwriters Laboratories approval is forbidden. Ground-fault interrupters are required for "wet" lab operators. Mobile laboratories must be properly grounded to protect against electrical shock.

E. Transportation

The operation of vehicles and boats shall be performed in accordance with established rules and regulations in the employees Field Safety Manual.

F. Emergency Health and Fire Protection

Upon arriving at the plant, inspectors should inquire about the availability of emergency ambulance service in case of personal injury or illness and fire protection for mobile labs and vehicles.

G. Accident Reports

EPA personnel shall report all job-connected accidents in accordance with procedures outlined in the EPA Occupational Safety and Health Manual.* Employees must furnish complete accident information to assure accurate reporting.

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*USEPA. 1977. Occupational Safety and Health Manual. Office of Planning and Management, U.S. Environmental Protection Agency, Washington, D.C.

Supervisors shall investigate and report within two working days all job-related accidents on EPA Form 144-9, Supervisor's Report of accident.

SECTION VII

CONDUCTING BIOMONITORING INSPECTIONS

A. Facilities Access

In obtaining consent to enter into a particular private facility for inspection and sampling purposes, the principles or rules an inspector should follow are:

1. Enter the permittee's premises through the main gate or through the entrance designated by the permittee in response to the inspection notification letter.

2. Advise the party at the gate or entrance that you wish to speak with the owner, lessee, person in charge or other person you have been instructed to contact.

3. Introduce yourself as an inspector to the owner, operator or agent in charge and advise that person that you wish to enter and perform the inspection and/or sampling you have been instructed to perform.

4. Present your official EPA or State credentials whether or not identification is requested.

5. Do not sign any "waivers" or "visitor releases" (U.S. EPA inspectors only) that purport to absolve the permittee of responsibilty for injury while on the premises.

6. Limit your conversations with the permittee and their employees or agents to obtaining directions to reaching sampling and inspection points and to making arrangements to split samples or making utility connections to the mobile lab.

7. If you are refused entrance, or are not allowed to complete your inspection or sampling tasks, immediately notify the person you have been instructed to notify in such circumstances. Follow instructions, such as to meet with the U.S. Attorney in the event that a decision is made to seek a warrant (see Appendix J, page 6).

B. Conducting Sampling-Type Inspections

1. On-site Testing

On-site testing may be performed using either a static or flow-through bioassay depending on the specific needs of the inspection. Appendix A of this manual, entitled "Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms" (EPA-600/14-78-012: Revised July 1978), contains methods and materials for bioassay testing which are referenced by EPA for compliance inspection purposes. Appendix A will be used to fulfill regulatory needs under section 304(h) of the Clean Water Act. Personnel performing compliance biomonitoring sampling inspections are referred to Appendix A for step-by-step procedures. An example of daily routine operations of an on-site biomonitoring sampling inspection is presented in Appendix D.

The inspector should avoid any alterations of EPA-approved bioassay methods and materials. Any such alteration could invalidate the results obtained from a bioassay. However, should such alteration be unavoidable, a corresponding and properly signed and dated logbook entry should be made at the time by the person(s) who introduced such alteration. Further, this logbook entry should also include detailed notes of the reasons for having had to introduce such alteration of methods and/or materials.

The NPDES Compliance Inspection Report (EPA Form 3560-3) should be used for all on-site inspections.

2. Off-site Testing

Off-site testing is most often performed using a 24-hour static bioassay. Any experienced member of an EPA inspection team can collect the effluent sample needed to conduct an off-site 24-hour static bioassay. Usually, the type of sample collected for this purpose is a grab sample, but occasionally a 24-hour composite sample may be collected depending on the nature of the effluent and the needs of the inspection.

The person collecting the sample should be supplied with the necessary tags and chain of custody forms. Appendix F supplies some examples of these types of tags and forms.

3. Sample Collection

Methods for collecting representative samples are covered in the NPDES Compliance Sampling Inspection Manual. Some important considerations for obtaining a representative sample are as follows:

(a) The sample should be collected where the wastewater is well mixed. The sample should be collected near the center of the flow channel, at 40-60% depth, where the turbulence is at a maximum and the possibility of solids settling is minimized. Skimming of the water surface or dragging the channel bottom should be avoided.

(b) In sampling from wide conduits, cross sectional sampling should be considered. Dye may be used as an aid in determining the most representative sampling point(s).

(c) The sampling of wastewater which contains immiscible liquids, such as oil and grease, requires special attention. Oil and grease may be present in wastewater as a surface film, an emulsion, or as a combination of these forms. As it is very difficult to collect a representative oil and grease sample, the inspector must carefully evaluate the location of the sampling point. The most desirable sampling location is the point where greatest mixing is occurring. Quiescent areas should be avoided, if possible. Because losses of oil and grease will occur on sampling equipment, the collection of a composite sample is impractical.

(d) If manual compositing is employed, the individual sample bottles must be thoroughly mixed before pouring the individual aliquots into the composite container.

C. Conducting Evaluation Inspections

1. Performance Audit Inspection (PAI)

This type of inspection involves observing, interviewing and recording pertinent information. An inspector with experience is a major ingredient in conducting a high-quality audit. An equally important ingredient is a properly completed NPDES Compliance Inspection Report (EPA Form 3560-3) and all other supporting inspection documentation.

a. On-site Inspection Responsibilities

Previously, in Section III, the inspector's responsibilities for planning and preparing a biomonitoring audit were discussed. The following information deals with on-site responsibilities of the inspector while conducting a biomonitoring audit.

It is the responsibility of the inspector to determine the conformance of the permittee's biomonitoring program with the corresponding permit and regulations. To accomplish this, the inspector should become thoroughly familiar with the permit's biomonitoring requirements and with any pertinent correspondence which may have modified permit conditions or procedures. Using a Biomonitoring Audit Form or a special notebook, the inspector should review all necessary documents and conduct a visual inspection of the permittee's sampling and testing procedures, biomonitoring equipment and facilities.

b. Effluent Sampling

The inspector is responsible for observing and auditing sampling practices as performed by the discharger. The following list contains important items which must be verified during a biomonitoring audit.

The inspector shall verify:

1) That samples are taken at the locations prescribed in the NPDES permit;

2) That the sampling location specified in the permit is adequate to provide a well mixed and representative sample;

3) That the frequency of sampling (grab samples and sampling interval for composites) is done in accordance with the NPDES permit;

4) That grab sample devices, if used, are clean and properly operated;

5) That containers are clean and appropriate for collecting samples;

6) That automatic sample collectors, if used, operate properly;

7) That chemical preservatives are not used in samples which are to be bioassayed;

8) That samples shipped to a contract laboratory

are properly preserved (refrigerated) and shipped in appropriate containers and under approved chain of custody procedures.

9) That samples are received and bioassay testing is initiated as soon as possible, but not later than 24 hours after sample collection.

10) That if there is more than one sampling point, a determination is made so appropriate measures are taken to prevent cross contamination between samples and that sample containers are properly identified;

11) That all testing equipment is routinely calibrated.

c. Laboratory Audit

On-site observation and audit of permittees' or their contractor's laboratory procedures, equipment and facilities should be performed by a well-trained inspector.

The following five major areas require detailed observations and evaluation. Under each listed item is a summary of "conditions" recommended by the EPA (see Appendix A).

1) Facilities

Effluent toxicity may be performed in a stationary or mobile laboratory. Depending upon the scope of the bioassay program, facilities may include equipment for rearing, holding and acclimating test organisms. Temperature control is achieved using circulating water baths, or environmental chambers. Holding, acclimation, and dilution water should be temperature-controlled and aerated whenever possible. Air used for aeration must be free of oil and fumes; filters to remove oil in water are desirable. Test facilities must be well ventilated and free of fumes. During holding, acclimating, and bioassay, test organisms should be shielded from outside disturbances.

Materials used in the construction of the test equipment which come in contact with the effluent should be carefully chosen. This is a must if one wishes to minimize leaching, dissolution, and adsorption (see Appendix A, pages 4-6).

2) Test Organisms

Inspectors should determine the source of the test organism. They must be familiar with the requirements of test organisms and review the handling and holding procedures used by the permittee or his agent.

3) Dilution Water

Dilution water may be ground water, surface water, reconstituted water, or dechlorinated tap water. Criteria for selecting and/or preparing dilution water are given in section 4, pages 14 to 16 of Appendix A.

The inspector should determine if the permittee's dilution meets these criteria.

4) Test Procedure

Perhaps the most important aspect of the biomonitoring audit consists of observing and carefully scrutinizing toxicity testing and related laboratory analyses as performed by the discharger or by a commercial laboratory under contract to the permittee.

5) Test Results

The inspector should have an in-depth understanding of chemical, physical and biological data required and the calculation methods used for interpreting biomonitoring test results. Data needs and methods for calculating LC50 and EC50 are presented in pages 24, 25 and 29 to 38 of Appendix A. Methods used for chemical analysis must be those specified in the final section 304(h) published as 40 CFR 136 regulations and Wastes, USEPA, EPA 600/4-79-020 March 1979, unless the specific NPDES permit has other requirements in which case they must be followed.

6) NPDES Compliance Inspection Report

Biomonitoring is part of the NPDES program. As such it shall use for reporting purposes the NPDES

Compliance Inspection Report (EPA form 3560-3). Additional information may be submitted using an evaluation form similar to that included in this manual as Appendix C - Acute Toxicity Laboratory Evaluation Form.

2. Compliance Evaluation Inspection

Prior to inspection, the inspector has become familiar with the permittee's operations, compliance track and self-biomonitoring activities.

In the compliance evaluation type of inspection, the inspector examines the production, waste treatment and laboratory facilities of the permittee. Files and records of the permittee's self-biomonitoring program are inspected. Additional guidance for these inspections may be found in the NPDES Compliance Evaluation Inspection Manual, July 1976.

3. Performance Audit Inspections

Depending on the needs of the enforcement agency, a performance audit inspection (PAI) of the permittee's records and procedures may be required. Guidance of performing PAIs is currently in draft form, but will be available from EPA HQ Office of Water Enforcement, Enforcement Division in the fall 1979.

Section VIII

Post Inspection Activities

A. Data Evaluation

The primary purpose of a Compliance Biomonitoring Inspection is to establish compliance status with bioassay requirements in an NPDES permit and/or to evaluate the effluents potential for toxicity to aquatic life in the receiving waters. This is accomplished by a thorough evaluation of compliance biomonitoring data, and is determined through a comparison of the following parameters:

1. Toxicity of the waste (LC50 or EC50)
2. Instream waste concentration (IWC) of the effluent.
3. Potential for chronic and acute toxicity of waste in the receiving water, including persistence, carcinogenicity, mutagenicity, and teratogenicity.
4. Permit limits, if contained in the permit.
5. Chemical parameters of effluent measured in conjunction with the bioassay such as D.O., temperature, pH, conductivity, metals, and organics.

The bioassay serves as a method for measuring the acute toxicity of an effluent. Because death of the test organisms is more easily observed and is less ambiguous than other physiological or behavioral effects used in determining toxicity, lethality is usually the endpoint in acute toxicity tests. Several procedures for evaluating lethality, expressed as LC50 (% effluent) are provided in the EPA manual on toxicity testing (Appendix A, pages 22-38).

The toxic effects that an effluent will have on aquatic organisms is greatly influenced by the degree of dilution that occurs in the receiving water. A commonly used parameter to describe this dilution is the instream waste concentration. The IWC is expressed as the volume percent of effluent in the combined effluent and receiving water and is defined by:

$$IWC = \left(\frac{Qw}{Qr + Qw}\right) \times 100$$

Where: IWC = Instream waste concentration (%)
Qw = Effluent flow (MGD)
Qr = 7Q10 (7 day, 10 year low flow of receiving waters in MGD)

The IWC is a theoretical value which assumes rapid and complete mixing of the effluent with the receiving water. However, the validity of this assumption depends on the specific hydrologic, geologic and morphologic aspects of the receiving water and basin. For example, in a small, turbulent stream, mixing may occur very rapidly, and the IWC could give a good indication of the effluent dilution.

However, in a larger stream or river, several distinct current systems may exist. In this case, the waste discharge will not mix evenly throughout the entire receiving stream, and will result in a plume of poorly diluted effluent. Similarly, in lakes and estuaries, mixing will occur fairly slowly, forming a mixing zone that contains effluent at a higher concentration than predicted by the IWC. It has been suggested, however, that the IWC can be applied to the boundary of the mixing zones in the lake. Yet even here the applicability is influenced by lake currents, the retention time of the lake waters, and the persistence of toxic pollutants from the effluent.

Although the IWC will not, in many cases, describe the dilution process precisely, it does provide a useful tool for approximating the dilution that will occur. Consequently, the IWC is frequently used in conjunction with LC50 or EC50 data, and an appropriate application factor to predict if a waste discharge may produce toxic effects on aquatic life in the receiving water. The application factors create a safety margin for setting water quality criteria to protect aquatic life and human health.

As supported by Water Quality Standards program guidance(1) and the recommendations of the 1972 NAS Report(2), an application

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1. Quality Criteria for Water, 1976. US EPA, Washington, D.C.
2. Water Quality Criteria, 1972. A report of the Committee on Water Quality Criteria. National Academy of Sciences - National Academy of Engineering, Washington, D.C.

factor of 0.01 for effluents containing persisitent or extremely toxic pollutants and an application factor of 0.5 for all other pollutants is recommended. The permittee is required to provide proof that his effluent does not contain known persistent, carcinogenic, mutagenic, or teratogenic substances in order to use the 0.5 application factor.

If the LC50, multiplied by the appropriate application factor is equal to or less than the calculated IWC, then the waste is considered to have the potential for being toxic to aquatic life in the receiving water. This comparison will serve as the basis for determining compliance with permit requirements.

	pass	fail
persistent	$0.01 \times \text{LC50} \geq \text{IWC}$	$0.01 \times \text{LC50} \leq \text{IWC}$
nonpersistent	$0.05 \times \text{LC50} \geq \text{IWC}$	$0.05 \times \text{LC50} \leq \text{IWC}$

Since water quality standards, in most cases, do not apply to mixing zones, to avoid acute toxicity in the immediate mixing zone it is recommended that an application factor of 0.33 should be used;

$$\text{LC50} = \frac{\text{IWC}}{0.33}$$ [1]

Chemical parameters which may have been measured during the course of the bioassay could be of assistance in interpreting the

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[1] This 0.33 application factor is based on experimental data collected and evaluated by EPA's Regions III and IV.

bioassay data. From this information, clues as to the cause of the toxicity can be obtained; and will aid in choosing corrective action.

Information on changes in waste characteristics should also be considered when evaluating toxicity problems.

B. Toxicity Laboratory Evaluation Form

A suggested format for recording pertinent information is presented in Appendix C. However, until such time as the current NPDES inspection report form is amended, the toxicity evaluation form should be included as an attachment to the regular inspection report form.

C. Distribution of Inspection Report

The NPDES Compliance Inspection Report (EPA form 3560-3) and any additional documentation obtained during inspection will be sent to enforcement personnel who are involved in permit writing and/or those who have compliance responsibilities. Further distribution of the report should be made by enforcement personnel only. Data and/or LC50, EC50, should be made available to personnel in charge of storing these data in the corresponding ADP systems (PCS, STORET).

D. Follow-up Activities

It is advisable that informational feedback mechanisms between enforcement personnel and compliance field inspection teams working on the same cases be instituted. This method of operation will ensure adequate case follow-up and maximum utilization of information and resources.

APPENDIX A

EPA-600/4-78-012
Revised July 1978

METHODS FOR MEASURING THE ACUTE TOXICITY OF EFFLUENTS TO AQUATIC ORGANISMS

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FOREWORD

Environmental measurements are required to determine the quality of ambient water, the character of effluents, and the effects of pollutants on aquatic life. The Environmental Monitoring and Support Laboratory-Cincinnati conducts research to develop, evaluate, and promulgate methods to:

- Measure the presence and concentration of physical, chemical and radiological pollutants in water, wastewater, bottom sediments, and solid waste.
- Concentrate, recover, and identify enteric viruses, bacteria, and other microorganisms in water.
- Measure the effects of pollution on freshwater, estuarine, and marine organisms, including the phytoplankton, zooplankton, periphyton, macrophyton, macroinvertebrates, and fish.
- Automate the measurement of the physical, chemical, and biological quality of water.
- Conduct an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

The Federal Water Pollution Control Act Amendments (Clean Water Act) of 1977 (PL 95-217) explicitly state that it is the national policy that the discharge of toxic substances in toxic amounts be prohibited. Determination of the toxicity of effluents, therefore, has high priority in the EPA water pollution control program. However, suitable, standardized methodology for effluent bioassays has not been available to EPA regional and state programs. This report fills an urgent current need for standardized methods to measure the toxicity of effluents to aquatic life.

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PREFACE

The effluent toxicity tests described in this document were prepared by the Bioassay Subcommittee of the EPA Biological Advisory Committee to provide methods needed for effluent monitoring by EPA Regional and State NPDES programs and for self-monitoring by NPDES permit holders.

Two types of toxicity tests are described:

1. A preliminary, short-term, static, range-finding (screening) test for use in determining the concentrations of effluent to be used in a "definitive" test.

2. A long-term (generally 96-hr), flow-through, definitive test for use in determining the acute toxicity of the effluent, expressed as a LC50 or EC50.

These methods will be included in the second edition of the EPA manual, "Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents," but were printed separately in limited quantity to make them available prior to the publication of the manual.

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ABSTRACT

This report describes methods for the measurement of the acute toxicity of effluents to macroinvertebrates and fish. The methods include a preliminary short-term (8-24 hr), range-finding (screening) test and a long term (96 hr.), flow-through (or alternate static) definitive test for use in determining the LC50 or EC50 of the effluent. The report includes guidelines on effluent sampling and holding, facilities and equipment, dilution water, test species selection and handling, and data interpretation.

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SECTION 1

INTRODUCTION

The Declaration of Goals and Policy of the Federal Water Pollution Control Act Amendments (Clean Water Act) of 1977 (PL 95-217), Section 101(a)(3), states that "it is the national goal that the discharge of toxic pollutants in toxic amounts be prohibited". Current Agency programs for the protection of aquatic life in receiving waters are based in part on effluent limitations for individual chemicals. However, toxicity data are available for only a limited number of compounds. Effluent limitations, therefore, may not provide adequate protection where the toxicity of the components in the effluent is not known, where there are synergistic effects between toxic substances in complex effluents, and/or where a complete chemical characterization of the effluent has not been carried out. Since it is not economically feasible to determine the toxicity of each of the thousands of potentially toxic substances in complex effluents or to conduct exhaustive chemical analyses of effluents, the most direct and cost-effective approach to the measurement of the toxicity of effluents is to conduct a bioassay with aquatic organisms representative of indigenous organisms in receiving waters. For this reason, the frequency of use of effluent bioassays to identify and control toxic discharges is rapidly increasing within the Agency and state NPDES programs, and by permittees as a self-monitoring requirement.

The lack of standardized bioassay methodology developed explicitly for effluents has delayed the implementation of EPA regional and state effluent toxicity testing programs and has resulted in a lack of uniformity in test procedures. In response to this problem, a subcommittee was organized within the EPA Aquatic Biology Methods Advisory Committee, sponsored by the Environmental Monitoring and Support Laboratory, Cincinnati, to prepare effluent bioassay methods for the NPDES program. To provide valid methods, it was essential to include EPA Regional and Enforcement programs personnel with extensive experience in conducting effluent toxicity tests. In completing their task, the subcommittee members drew from their own experience and borrowed heavily from the report, "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians," (EPA 660/3-75-009), previously prepared primarily to standardize basic laboratory methods for determining the toxicity of pure compounds.

The acute toxicity tests for effluents described in this report are used to determine the effluent concentration, expressed as a percent volume, that is lethal to, or has some other "adverse effect" on, 50 percent of the organisms within 96 hrs or some other prescribed period of time. If mortality (death) is the effect measured, the toxicity is expressed as the median lethal concentration or LC50. Where death is not easily detected, for example, with some invertebrates, other indicators such as immobilization must be used as the adverse effect. Blood chemistry, biochemical measurements or histological examinations can also be employed to determine the toxicity of an effluent. The concentration of effluent, expressed as a percent volume, that causes a defined adverse effect other than death in 50 percent of the test organisms within the prescribed exposure period is termed the median effective concentration or EC50.

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A two-test sequence is generally required to estimate the acute toxicity of an effluent: (1) a preliminary, short-term (8-24 hr), range-finding test is conducted to define the range of effluent dilutions to be used in the definitive test, and (2) a more rigorous long-term definitive test is conducted (using the range of effluent dilutions determined by the range-finding test) over a 96-hour time period to arrive at the acute toxicity of the effluent, which is expressed as a LC50 or EC50.

One of the following procedures shall be used for the range-finding test:

A. Test organisms are placed in suitable containers and exposed under static conditions to 3-5 widely-spaced dilutions of the effluent for a period of 8-24 hours.

B. If the effluent has a high dissolved oxygen demand, or the organisms require flowing water, a flow-through test is used to define the range of toxicity of the effluent.

One of the following procedures shall be used for the definitive test:

A. Preferred procedures (flow-through tests), in order of preference

1. Test organisms are exposed to effluent solutions flowing into and out of test chambers on a once-through basis for the duration of the test. The effluent is conveyed directly and continuously from the source to the dilutor system.

2. Test organisms are exposed to effluent solutions flowing into and out of test chambers on a once-through basis for the duration of the test as in A.1 above. However, the effluent is supplied to the dilutor system from discrete effluent samples collected periodically. The interval at which samples are collected is based on the variability of the effluent characteristics, production schedule, batch processes, retention time, etc. (see p. 17 - Effluent Sampling and Holding).

The continuous effluent sampling technique (A.1) is the best of two methods described.

B. Alternative procedures (static tests), in order of preference

1. Test organisms are exposed to a fresh solution of the same concentration of effluent every 24 hours, either by transferring the test organisms from one test chamber to another, or by replacing the effluent solution in the test chambers.

2. Test organisms are exposed to the same effluent solution for the duration of the test. However, the effluent solution in

each test chamber may be filtered, aerated, or sterlized by continuous circulation through an appropriate apparatus.

3. Test organisms are exposed to the original effluent solution for the duration of the test without being continuously circulated through an apparatus as in B.2.

The alternative procedures may be used in emergency situations or where adequate facilities are not available to the investigator. However, it must be understood that a test using the alternative procedures is not valid unless it can be conclusively demonstrated that the chemical characteristics and toxicity of the effluent do not change over time. Because of toxicant adsorption on the test chambers, uptake by test organisms and the effect of metabolites on toxicity, it is preferred that the effluent solution be renewed at least once every 24 hours as described in B.1 above.

The special environmental requirements of some organisms, such as flowing water, fluctuating water levels, or substrate may preclude the use of static testing.

SECTION 2

FACILITIES AND EQUIPMENT

GENERAL REQUIREMENTS

Effluent toxicity tests may be carried out in a fixed or mobile lab. Depending upon the scope of the bioassay program, facilities may include equipment for rearing, holding and acclimating organisms. Temperature control is achieved using circulating water baths, or environmental chambers. Dilution water may be ground water, surface water, reconstituted water, or dechlorinated tap water. Holding, acclimation, and dilution water should be temperature-controlled and aerated whenever possible. Air used for aeration must be free of oil and fumes; filters to remove oil in water are desirable. Test facilities must be well ventilated and free of fumes. During holding, acclimating, and testing, test organisms should be shielded from disturbances.

Some organisms may have special environmental requirements such as flowing water, fluctuating water levels, or substrate which must be provided. During holding, acclimating, and testing, immature stream insects should always be in flowing water; as described by Nebeker and Lemke (1968); penaeid shrimp and bottom-dwelling fish should be provided a silica sand substrate. Since cannibalism can occur among many species of arthropods, they should be isolated by some means (e.g., with screened compartments), or the claws of crabs and crayfish should be bound.

CONSTRUCTION MATERIALS

Materials used in the construction of the test equipment which come in contact with the effluent should be carefully chosen. Glass, No. 316 stainless steel, and perfluorocarbon plastics (TEFLON^R) should be used whenever possible to minimize leaching, dissolution, and sorption. Linear polyethylene may also be used with some types of industrial and municipal effluents, but should be avoided with those containing synthetic organic compounds or pesticides. Unplasticized plastics such as polyethylene, polypropylene, TYGON^R and fiberglass can be used for holding, acclimating, and dilution-water storage tanks, and in the water delivery system. Copper, galvanized material, rubber, brass, and lead must not come in contact with holding, acclimation, or dilution water, or with effluent samples and test solutions.

EFFLUENT (TOXICANT) DELIVERY SYSTEM (Flow-through test only)

Although many types of toxicant delivery systems have been designed*, the flow-through, proportional-dilutor delivery system has proven to be the best and the preferred system for routine effluent toxicity tests conducted in both fixed and mobile laboratories.

The following factors should be considered in designing the system: (1) whether the apparatus will be installed and used in a fixed or mobile laboratory; (2) the existence of adequate space and/or structural requirements for the delivery system, test chambers, effluent and dilution-water storage; (3) the applicability of the delivery system to specific effluent characteristics (high suspended solids, volatiles, etc.); (4) the system's dependability, durability, flexibility, and ease of maintenance and replacement; (5) the ability to perform within the flow rate and concentration accuracy limitations; and (6) the cost of the system.

Two types of proportional dilutors are described in the appendix - the solenoid valve system and the vacuum siphon system. The solenoid valve system is preferred, but the vacuum siphon system is acceptable, if funds are limited.

The flow rate through the proportional dilutor must provide for at least five complete water volume changes in 24 hours in each test chamber, plus sufficient flow to maintain an adequate concentration of dissolved oxygen. It is often desirable to construct the dilutor with an additional reserve flow capacity, depending on its application and/or special effluent characteristics. The flow rates through the test chambers should not vary by more than 10 percent among test chambers at any time during any test. The dilutor should also be capable of maintaining the test concentration in each test chamber within 5 percent of the starting concentration for the duration of the test.

The calibration of the dilutor should be checked carefully before and after each test. This check should determine the volume of effluent and dilution water used in each portion of the effluent delivery system and the flow rate through each test chamber. The general operation of the dilutor should be checked at least at the beginning and end of each day during the test.

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*(Lowe, 1964; Mount and Brungs, 1967; Sprague, 1969; Freeman, 1971; Cline and Post, 1972; Granmo and Kollberg, 1972; Bengtsson, 1972; Lichatowich, *et al.*, 1973; Schumway and Palensky, 1973; Abram, 1973; Schimmel, Hansen, and Forester, 1974; DeFoe, 1975; Riley, 1975).

TEST CHAMBERS

Type

Test chambers used in flow-through tests are constructed of 1/4 inch plate glass held together with (GE) clear silicone adhesive. Silicone adhesive absorbs some organochlorine and organophosphorus pesticides, which are then difficult to remove. Therefore, as little of the adhesive as possible should be in contact with water; extra beads of adhesive inside the containers should be removed. Stainless steel (#316) can be used in the construction of test chambers, but must be of welded, not soldered, construction.

The size of the chambers may vary according to the size of the test organism and/or the facilities, but the test solution should have a minimum depth of 5 cm. All chambers should have either a glass or screen cover to prevent organisms from jumping out.

The test chambers most commonly used in static tests are wide-mouth, 3.9 liter (1-gallon) or 19.6 liter (5-gallon) soft-glass bottles. Containers such as 1-liter battery jars or 250-ml beakers may be more suitable as test chambers for fish eggs and/or larvae and small crustacea. Special glass or stainless steel test chambers can be constructed to accommodate test organisms requiring special physical conditions.

Cleaning

All test chambers, whether new or used, must be washed in the following manner to remove surface contaminants:

A. Soak and wash in suitable detergent in water, preferably heated to a temperature of 50°C or higher. The detergent (powder or liquid) should be entirely synthetic (SPARKLEEN^{R} or ALCONOX^{R}).

B. Rinse with water (preferably heated to 50°C or higher).

C. Rinse with a fresh, dilute (5 percent) hydrochloric acid for removing metals and bases.

D. Rinse with water (preferably heated to 50°C or higher).

E. Rinse with acetone to remove organic compounds. When contaminated with a pesticide, test chambers must be rinsed with acetone before they are placed in the hot detergent soak (Item A. above).

F. Rinse twice with water.

When feasible, the outlined cleaning procedure must be used for other equipment that comes in contact with the dilutor system, pumps, tanks, etc. All test chambers and equipment must be thoroughly rinsed with the dilution water prior to each test.

SECTION 3

TEST ORGANISMS

SPECIES

Whenever possible, effluent toxicity tests should be conducted with a sensitive species that is indigenous to the receiving water, readily available, and either commercially or recreationally important. Acceptable species include those listed in Table 1, which are sensitive to most toxicants. The test organisms must be identified to species.

SOURCE

Although effluent tests should be conducted with species that are indigenous to, or stocked into, the receiving water, the test organisms do not have to be taken from the receiving water. It is often difficult to obtain fish of the desired size and condition from the receiving water. Collection permits are often difficult to obtain, and the organisms in the receiving water may have been previously exposed to the effluent. Fish captured by electroshocking must not be used in testing. The usual sources of freshwater fish used for toxicity tests are private, state, and Federal hatcheries. If trout are used as test organisms, they should be obtained from stock that has been certified as disease-free.

Some fish, such as Fathead and Sheepshead minnows, are easily reared under laboratory conditions (USEPA, 1972; Schimmel and Hansen, 1974). However, it is usually more practical to collect marine species from indigenous populations.

Some invertebrates such as daphnids, midges, and shrimp may be reared in laboratory cultures. Care must be taken to insure that only young age groups and early instars are used in testing. Daphnids from cultures in which ephippia are being produced should not be used in the tests. Invertebrates not amenable to laboratory rearing are usually obtained directly from wild populations.

SIZE

Very immature fish (not actively feeding on exogenous food), spawning fish, or recently spent fish should not be used. Fish weighing between 0.5 and 5.0 grams each are preferred. In any single test, all fish should be taken from the same year class, and the total length (tip of snout to end of tail) of the longest fish should be no more than 1-1/2 times that of the shortest one. As stated above, immature invertebrates should be used whenever possible.

TABLE 1. RECOMMENDED SPECIES AND TEST TEMPERATURES

Species	Test Temperature (°C)[a]
Freshwater	
Vertebrates	
Coho salmon, *Oncorhynchus kisutch*	12
Rainbow trout, *Salmo gairdneri*	12
Brook trout, *Salvelinus fontinalis*	12
Goldfish, *Carassius auratus*	22
Fathead minnow, *Pimephales promelas*	22
Channel catfish, *Ictalurus punctatus*	22
Bluegill, *Lepomis macrochirus*	22
Invertebrates[a]	
Daphnids, *Daphnia magna* or *D. pulex*	17
Amphipods, *Gammarus lacustris*, *G. fasciatus*, or	17
G. pseudolimnaeus	17
Crayfish, *Orconectes* sp., *Cambarus* sp., *Procambarus*	22
sp., or *Pacifastacus leniusculus*	22
Stoneflies, *Pteronarcys* sp.	12
Mayflies, *Baetis* sp. or *Ephemerella* sp.	17
Hexagenia limbata or *H. bilineata*	22
Midges, *Chironomus* sp.	22
Marine and estuarine	
Vertebrates	
Sheepshead minnow, *Cyprinodon variegatus*	22
Mummichog, *Fundulus heteroclitus*	22
Longnose killifish, *Fundulus similis*	22
Silverside, *Menidia* sp.	22
Threespine stickleback, *Casterosteus aculeatus*	22
Pinfish, *Lagodon rhomboides*	22
Spot, *Leiostomus xanthurus*	22
Shiner perch, *Cymatogaster aggregata*	12
Pacific staghorn sculpin, *Leptocottus armatus*	12
Sanddab, *Citharichthys stigmaeus*	12
Flounder, *Paralichthys dentatus*, *P. lethostigma*	22
English sole, *Parophrys vetulus*	12

TABLE 1. (Cont'd)

Species	Test Temperature (°C)[a]
Marine and estuarine	
Invertebrates[a]	
Shrimp, *Penaeus setiferus*, *P. duorarum*, or *P. aztecus*	22
Grass shrimp, *Palaemonetes* sp.	22
Shrimp, *Crangon* sp.	22
Oceanic shrimp, *Pandalus jordani*	12
Blue crab, *Callinectes sapidus*	22
Dungeness crab, *Cancer magister*	12
Mysid shrimp, *Mysidopsis* sp., *Neomysis* sp.	22
Atlantic oyster, *Crassostrea virginica*	22
Pacific oyster, *Crassostrea gigas*	20

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[a] Freshwater amphipods, daphnids, and midge larvae and shrimp should be cultured and tested at the recommended test temperature. Other invertebrates should be held and tested within 5°C of the temperature of the water from which they were obtained. If the recommended test temperature is not within this range, they should be tested at the temperature from the series 7, 12, 17, 22, and 27°C that is closest to the recommended test temperature and is within the allowed range.

HOLDING AND HANDLING

Disinfect holding tanks or chambers with 0.5 percent commercial bleach for one hour. Brush thoroughly with the disinfectant. Rinse well between groups of organisms. Other equipment used to handle organisms must be disinfected with 0.5 percent commercial bleach (5 ml of bleach added to 1 liter of water), or 30-percent formalin.

When feasible, holding tanks must receive an uncontaminated, constant-quality water in a flow-through system with a flow rate of at least 2 tank-volumes per day. Otherwise, a recirculation system where the water flows through a charcoal filter to remove dissolved metabolites or passes by an ultra-violet light for disinfection is necessary. Only as a last resort should a dechlorinated tap water be used for freshwater organisms or a synthetic salt water for marine organisms.

When organisms are first brought into a facility, they must be quarantined for a minimum of 10 days. To avoid unnecessary stress after collection and transportation, organisms should not be subjected to a change of more than a 3°C in water temperature or 3-ppt in salinity in any 12-hour period, or a total change of not more than 6°C or 6 ppt salinity. Invertebrates should be held within 5°C of the temperature of the water from which they were obtained. To maintain organisms in good condition during holding, crowding should be avoided. The dissolved oxygen concentration must be greater than 40 percent of saturation for warm water species and greater than 60 percent of saturation for cold water species. Aeration may be used if necessary.

Organisms should be fed at least once a day, and excess food and fecal material should be removed from the bottom of the tanks at least twice a week by siphoning. Organisms should be observed carefully for signs of disease, stress, physical damage, and mortality. Dead and abnormal specimens should be removed as soon as observed. A daily log of feeding, behavioral observations, and mortality must be maintained.

Organisms should be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and quickly as possible to minimize stress. Organisms that touch dry surfaces or are dropped or injured during handling must be discarded. Dipnets are best for handling larger organisms. Such nets are commercially available or can be made from small-mesh nylon netting, nylon or silk bolting cloth, plankton netting, or similar material. Nets coated with urethane resin are best for handling catfish. Smooth glass tubes with rubber bulbs should be used for transferring smaller organisms such as daphnids and midge larvae.

DISEASE TREATMENT

During holding, fresh and salt water fish should be chemically treated to cure or prevent disease as recommended in Table 2. However, if the fish are severely diseased, it is advisable to discard the entire lot. When invertebrates become diseased, they should be discarded. Tanks which are contaminated with disease-causing microorganisms must be disinfected with 0.5 percent commercial bleach.

TABLE 2. RECOMMENDED PROPHYLACTIC AND THERAPEUTIC TREATMENTS FOR FRESHWATER FISH[a]

Disease	Chemical	Concentration (mg/l)	Duration of[c] Treatment
External Bacteria	Oxytetracycline hydrochloride (water soluble)	25[b]	30-60 min
	Procaine Penicillin G in Dihydrostreptomycin sulfate solution (Franklin Lab, Denver,CO)	(3ml/100 gal)	48-72 hrs
	Benzalkonium chloride (HYAMINE 1622®)	1-2[b]	30-60 min
	Nitrofurazone (water mix)	3-5[b]	30-60 min
	Neomycin sulfate	25	30-60 min
Monogenetic trematodes fungi, and external protozoa[d]	Formalin plus zinc-free malachite green oxalate	25 0.1	1-2 hrs
	Formalin	150-250	30-60 min
	Potassium permanganate	2-6	30-60 min
	Sodium chloride	15000-30000 2000-4000	5-10 min dip (e)
	DEXON® (35% Active Ingredient)	20	30-60 min
Parasitic copepods	Trichlorfon (MASOTEN®)	0.25[b]	Continuous[f]

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[a] This table indicates the order of preference of treatments that have been reported to be effective, but their efficacy against diseases and toxicity to fish may be altered by temperature or water quality. Caution: test treatments on small lots of fish before making large-scale applications. Fish should not be treated the first day they are in the facility.

TABLE 2 (continued).

Before using a treatment other than those listed in this table, additional information should be obtained from sources such as Davis (1953), Hoffman and Meyer (1974), Reichenbach-Klinke and Elkan (1965), Snieszko (1970), and van Duijn (1973).

b. Active ingredient.

c. Treatment may be accomplished by (1) transferring the fish to a static treatment tank and back to a holding tank; (2) temporarily stopping the flow in a flow-through system, treating the fish in a static manner, and then resuming the flow to flush out the chemical; or (3) continuously adding a stock solution of the chemical to a flow-through system by means of a metered flow or the technique of Brungs and Mount (1967).

d. One treatment is usually sufficient except for Ichthyophthirius ("Ich"), which must be treated daily or every other day until no sign of the protozoan remains. This may take 4-5 weeks at 5-10°C and 11-13 days at 15-21°C. A temperature of 32°C is lethal to "Ich" in one week.

e. Minimum of 24 hours, but may be continued indefinitely.

f. Continuous treatment should be employed in static or flow-through systems until no copepods remain, except that treatment should not be continued for more than 4 weeks and should not be used above 27°C.

TRANSPORTATION AND ACCLIMATION

Organisms reared and held at a fixed facility are transported to the test site in the water in which they were reared and held. If the laboratory is mobile, the acclimation tank can be used in transporting organisms from the rearing and holding facilities to the test site. At the test site, dilution water (receiving water) is pumped to the laboratory for use in the acclimation of the organisms. If dilution water is not readily accessible, it can be transported to the laboratory and stored in a tank for use in the acclimation procedure and toxicity test. During transport and acclimation the organisms should not be subjected to a change of more than 3°C in water temperature or 3-ppt in salinity in any 12-hour period, or a total change of not more than 6°C or 6-ppt salinity, and the concentration of dissolved oxygen must not fall below 40 percent of saturation for warm water species and 60 percent of saturation for cold water species.

Upon arriving at the test site, the organisms are acclimated to the test dilution water and temperature by gradually changing from 100-percent holding water to 100-percent dilution water over a period of 24 hours. All organisms must be exposed to 100-percent dilution water for at least 24 hours before they are used for the tests, and must be held at the test temperature ($\pm$2°C) for at least 24 hours before tests are begun.

A group of organisms must not be used for a test if they appear to be diseased or otherwise stressed, or if more than 5 percent die during the 48 hours immediately preceding the test. If the organisms fail to meet these criteria, the entire group must be discarded and a new group obtained. The same acclimation procedure must be followed for the new group or ogranisms. Mortality of the test organisms during the acclimation period may be due to the presence of toxicants in the dilution water (receiving water). If excessive mortality occurs during the acclimation of the second group of test organisms, an alternate source of dilution water must be used.

The acclimation of marine organism for effluent toxicity tests poses special problems because most effluents discharged into the marine environment consist of adulterated freshwater. Therefore, when the effluent is diluted with the receiving water (salt water), the higher percent effluent volumes will have a low salinity (the salinity will be inversely proportional to the percent volume of effluent). If the effluent is essentially freshwater, it is obvious that 100% effluent cannot be used with the marine test organisms. The highest effluent concentration (lowest salinity) tested will depend upon the salinity of the receiving water and the tolerance of the test organism. (Sheepshead minnows and mysid shrimp are known to be tolerant to a salinity range of 5-35 ppt, but the tolerance of other marine species in Table 1 must be established by the investigator). Under the circumstances described above, it will be necessary to acclimate marine organisms to a series of salinities, ranging from 5-35 ppt. It would also be advisable to culture the test organisms at a series of salinity levels, including at least 10, 20, and 30 ppt, so that changes in salinity upon acclimation do not exceed 6 ppt.

SECTION 4

DILUTION WATER

A dilution water is acceptable if healthy test organisms survive in it through the acclimation period and toxicity test without showing signs of stress, such as discoloration or unusual behavior. For effluent toxicity testing, the dilution water should be a representative sample of the receiving water, and should be obtained from a point as close as possible to, but upstream of or outside of, the zone influenced by the effluent. It is preferable to pump the dilution water continuously to the acclimation tank and dilutor. However, it may be more practical to transport batches of water in tanks to the testing site as the need arises, and then continuously pump water to these systems.

In an estuarine environment, the investigator should collect uncontaminated water having a salinity as near as possible to the salinity of the water at the receiving site.

Pretreatment of the dilution water should be limited to filtration through a nylon sieve having 2-mm or larger holes to remove debris and/or break up large floating or suspended solids. The water should be obtained from the receiving water as close as possible to the time the test begins. It should not be obtained more than 96 hours prior to testing. If acceptable dilution water cannot be obtained from the receiving water, some other uncontaminated, well-aerated surface or ground water, or commercially available media, can be used. This water must have a total hardness, total alkalinity, and specific conductance within 25 percent, and pH within 0.2 units, of the receiving water at the time of testing.

If the substitute dilution water must be modified, reconstituted water must be prepared for use as the diluent. Recommended procedures are given in Tables 3 and 4. There are also commercially available salt water media such as INSTANT OCEAN^{R} and RILA SALTS^{R}.

With highly toxic effluents requiring very large volumes of dilution water, it may be convenient to locate the testing facility near the source of the dilution water, and transport the effluent.

TABLE 3. PREPARATION OF RECONSTITUTED FRESH WATERS [a]

3a. Quantities (mg/l) of reagent grade chemicals required to prepare recommended reconstituted fresh waters and the resulting water qualities.

Water Type	Reagent Added				Final Water Quality		
	$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KCL	pH[b]	Hardness[c]	Alkalinity[c]
Very soft	12	7.5	7.5	0.5	6.4-6.8	10-13	10-13
Soft	48	30.0	30.0	2.0	7.2-7.6	40-48	30-35
Hard	192	120.0	120.0	8.0	7.6-8.0	160-180	110-120
Very hard	384	240.0	240.0	16.0	8.0-8.4	280-320	225-245

3b. Quantities of reagent-grade chemicals to be added to aerated, soft reconstituted freshwater for buffering pH. The solutions should not be aerated after addition of these chemicals.

pH[d]	Volume (ml) of solution added to 15 liters of water		
	1.0N NaOH	1.0 m KH_2PO_4	0.5 m H_3BO_3
6.0	1.3	80.0	-----
6.5	5.0	30.0	-----
7.0	1[illegible]0	30.0	-----
7.5	---	----	-----
8.0	19.0	20.0	-----
8.5	6.5	----	40.0
9.0	8.8	----	30.0
9.5	11.0	----	20.0
10.0	16.0	----	18.0

a. From Marking and Dawson (1973).
b. Approximate equilibrium pH after aeration and with fish in water.
c. Expressed in mg/l as $CaCO_3$.
d. Approximate equilibrium pH with fish in water.

TABLE 4. PREPARATION OF RECONSTITUTED SEA WATER[a,b]

Add the following reagent-grade chemicals in the amounts and order listed to 890 ml distilled water. Each chemical must be dissolved before another is added.

Chemical	Amount
NaF	3 mg
$SrCl_2 \cdot 6H_2O$	20 mg
H_3BO_3	30 mg
KBr	100 mg
KCl	700 mg
$CaCl_2 \cdot 2H_2O$	1.47 g
Na_2SO_4	4.00 g
$MgCl_2 \cdot 6H_2O$	10.78 g
NaCl	23.50 g
$Na_2SiO_3 \cdot 9H_2O$	20 mg
Na_4EDTA(c)	1 mg
$NaHCO_3$	200 mg

a. If the resulting solution is diluted to 1 liter, the salinity should be 34±0.5 g/kg, and the pH 8.0±0.2. The desired salinity is attained by dilution at time of use.

b. From Kester *et al*. (1967), Zaroogian *et al*. (1969), and Zillioux *et al*. (1973).

c. Tetrasodium ethylenediaminetetraacetate. This coumpound should be omitted when toxicity tests are conducted with metals. When tests are conducted with plankton or larvae, the EDTA should be omitted and the medium should be stripped of trace metals (Davey *et al*., 1970).

SECTION 5

EFFLUENT SAMPLING AND HOLDING

SAMPLING

The effluent sampling point must be the same as that specified in the NPDES discharge permit. Conditions for exception would be: (1) better access to a sampling point between the final treatment and the discharge outfall, or (2) if the processed waste is chlorinated prior to discharge to the receiving waters, the sampling point may be located prior to contact with the chlorine if the purpose of the test is to determine toxicity levels of the unchlorinated effluent. Sampling should be based on an understanding of the short and long-term operations and schedules of the discharger. It is desirable to evaluate an effluent sample that most closely represents the "normal" or "typical" discharge and operating conditions of the plant in question. The retention time of the effluent in the waste water treatment facility, as indicated in Par. A.1.a-c and B.1-3 below, must be measured using dye studies. The only way in which the sample may be altered prior to testing is by filtering through a TEFLON^R or stainless steel screen with 2-mm or larger holes.

A. Flow-through test

1. If the industrial or municipal facility discharges continuously, the effluent should be pumped directly and continuously from the discharge line to the dilutor system for the duration of the test. The use of the effluent grab samples should be avoided. However, if the effluent cannot be pumped directly and continuously to the dilutor system, the following alternative methods may be employed for collection of the effluent:

    a. When the measured minimum retention time of the effluent is less than 96 hours, as determined from dye studies, a 6-hour composite sample, consisting of equal volumes taken every 30 minutes, must be collected and transported to the dilutor every 6 hours for the duration of the test.

    b. When the measured minimum retention time of the effluent is between 4 days (96 hours) and 14 days, as determined from dye studies, then a 24-hour composite sample, consisting of equal volumes taken every hour, may be collected daily for the duration of the test.

c. When the measured minimum retention time of the effluent is greater than 14 days, as determined from dye studies, a single grab sample may be collected daily for the duration of the test.

2. If the industrial or municipal facility discharges intermittently (i.e. where the waste is discharged over a single 8-hr work shift, or is accumulated and discharged at the end of the shift, or end of the week), a composite sample, consisting of equal volumes collected every 30 minutes, may be taken for an 8-hr operating shift or for the duration of the plant operating schedule, or a single grab sample may be taken in the case of a batch discharge.

B. Static test

If a flow-through test cannot be used, a static test may be conducted with effluent collected by one of the following methods:

1. When the measured minimum retention time of the effluent is less than 96 hours, as determined with dye studies, four consecutive 6-hr composite samples, each consisting of equal volumes taken every 30 minutes, are collected and used in setting up 4 separate static tests.
2. When the measured minimum retention time of the effluent is between 4 days (96 hours) and 14 days, as determined with dye studies, a 24-hour composite sample, consisting of equal volumes taken every hour, is collected daily and used in the test.
3. When the measured minimum retention time of the effluent is greater than 14 days, as determined with dye studies, a single grab sample may be collected and used in the test.

HOLDING

Effluent grab samples must be stored in covered, unsealed containers. Violent agitation must be avoided. However, undissolved materials must be uniformly dispersed by gentle agitation. This agitation must immediately precede adjustment of any aliquot of the effluent to test temperatures before adding it to the dilution water. Although it is desirable to refrigerate samples prior to the test, it is often convenient to store samples in a constant-temperature water bath or controlled-environment room at the temperature at which the test is conducted. The test should be initiated as soon as possible, but no later than 24 hours after collection of the effluent.

The persistence of the toxicity of an effluent may be a factor in determining specific toxicity limits in an NPDES permit, and is determined by measuring its toxicity upon collection and again after holding 96 hours. If after holding the effluent 96 hours, its toxicity has not decreased 50% or more, it is classified as persistent. (When special tests, such as persistence are conducted, the exact methodology must be detailed in the report.)

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SECTION 6

TEST PROCEDURE

RANGE-FINDING(SCREENING) TEST

Unless the approximate toxicity of the effluent is already known, it is necessary to conduct an abbreviated, preliminary, range-finding or screening test to determine the concentrations that should be used in the definitive tests. This test can be either a static or flow-through test. However, the test most often used is an abbreviated static test in which groups of 5 organisms are exposed to three to five widely-spaced effluent dilutions, and a control, for 8 to 24 hours.

Because the characteristics of the effluent and the receiving water may vary significantly within short periods of time, the toxicity observed in a range-finding test may not be representative of the toxicity of the effluent. If the range-finding test is to be conducted with the same sample of the effluent with which the definitive test is to be conducted, the duration of the range-finding test cannot exceed 24 hours (see limits in holding time for effluents, p. 18).

DEFINITIVE TEST

Test Conditions

The determination of a LC50 or EC50 must employ a control and at least five concentrations of effluent in an exponential series. To calculate the LC50 or EC50 with reasonable accuracy, a definitive test must meet both of the following criteria:

A. Each concentration of the effluent must be at least 50 percent of the preceding concentration.

B. One concentration must have killed (or affected) more than 65 percent of the organisms exposed to it, and one concentration other than the control must have killed (or affected) less than 35 percent of the organisms.

If 100-percent effluent does not kill (or affect) more than 65 percent of the organisms exposed to it, the percentage of organisms killed (or affected) by various levels of the effluent in the receiving water must be reported.

The control shall consist of the same dilution water, conditions, procedures, and organisms used in testing the effluent. A test is not acceptable if more than ten percent of the organisms die in the control.

Number of Test Organisms

At least 20 organisms of a given species must be exposed to each treatment. More than one species may be used in the same test chamber in a given test, if segregated. One-half of the organisms of each species exposed to each treatment should be placed in separate test chambers to serve as replicates. To qualify as true replicates, no water connections can exist between replicate test chambers. Randomization of treatments is desirable.

Test animals are normally captured for transfer from acclimation tanks to test chambers by dip netting. No more than 20 percent of the total number of organisms transferred to each chamber should be added from a given net capture.

Loading of Test Organisms

For all tests, a limit must be placed on the weight of organisms per liter of test solution. This practice will minimize the depletion of dissolved oxygen, the metabolic conversion of effluent constituents, the accumulation of metabolic waste products, and/or stress induced by crowding, any of which could significantly affect the test results.

For flow-through tests, loading in the test chambers must not exceed 5 grams per liter at temperatures of 20°C or less, or 2.5 grams per liter at temperatures above 20°C.

For static tests, loading in the test chambers must not exceed 0.8 grams per liter at temperatures of 20°C or less and 0.4 grams per liter at temperatures above 20°C.

Test Temperature

For flow-through tests, it is desirable to hold the temperature within ±2.0°C of the acclimation temperature throughout the test. This can be accomplished by passing the effluent and/or dilution water through separate stainless steel coils immersed in a heating or cooling water bath prior to entering the dilutor system.

For static tests, the temperature may be that at which the test organisms were held prior to transportation or acclimation at the site. The instantaneous ambient temperature should not vary more than ±2°C at any time during the test.

Dissolved Oxygen

Aeration may alter the results of toxicity tests and, as a general rule, should not be employed. It can reduce the apparent toxicity of an effluent by stripping it of highly volatile toxic substances, or increase its toxicity by altering the pH. However, the dissolved oxygen concentration (DO) in the test solution should not be permitted to fall below 40 percent saturation for warm water species and 60 percent saturation for cold water species. In most flow-through tests, DO depletion is not a problem in the test chambers because aeration occurs as the liquids pass through the dilutor system.

If the DO concentration decreases to a level that would be a source of additional stress, the turnover rate of the solutions in the test chambers must be increased sufficiently to maintain acceptable DO levels. If the increased turnover rate does not maintain adequate DO levels, aerate the dilution water prior to the addition of the effluent, and aerate all test solutions.

Caution must be exercised to avoid excessive aeration, and it should be used only as a last resort in maintaining adequate DO levels. When aeration is used, the exact methodology must be detailed in the report.

Beginning the Test

The test begins when the test organisms are first exposed to the effluent.

A. Flow-through test

The dilutor system should be in operation 24 hours prior to the addition of the test organisms and the beginning of the test. During this period, necessary adjustments can be made in the percent effluent volumes, temperature, and flow rate through the test chambers.

B. Static test

The effluent is added to the dilution water and mixed well by stirring with a glass rod. The test organisms are placed in the chambers within 30 minutes. This procedure conserves DO and is sufficient for the effluent to become evenly dispersed in the dilution water.

Feeding

Organisms should not be fed during the test unless they are newly hatched or very young. In the case of fish, feeding should be terminated 48 hrs before the beginning of the test. Problems caused by feeding, such as the possible alteration of toxicant concentration, the build-up of food and metabolic wastes and resulting oxygen demand, are common in static test systems, but are minimal in flow-through systems.

Duration

The test duration may range from a minimum of 8 hours to 96 hours, depending on the test organism used, the purpose of the test and whether it is a range-finding test or a definitive test.

SECTION 7

TEST RESULTS

BIOLOGICAL DATA

The lengths and weights of the test organisms should be determined by sacrificing and measuring representative organisms before the test or by obtaining the lengths and weights of all surviving organisms at the end of the test. The number of dead (or affected) organisms in each test container should be counted 24, 48, 72, and 96 hours after the beginning of the test. (See data sheet in Appendix, Fig. 1, p. 33). Dead organisms must be removed at least once every 24 hours.

Death is the "effect" most frequently used for determining toxicity to aquatic organisms. The criteria usually employed in establishing death are: (1) no movement (especially no gill movement in fish), and (2) no reaction to gentle prodding. Death is not easily determined for some invertebrates, and some other effect often must be used. The effect generally used for determining toxicity to daphnids and midge larvae is immobilization, which is defined as the inability to move except for minor activity of appendages. With crabs, crayfish, and shrimp, the effects used are immobilization and loss of equilibrium. Other effects can be used for determining an EC50, but the effect and its definition must always be reported. General observations on such things as erratic swimming, loss of reflex, discoloration, changes in behavior, excessive mucus production, hyper-ventilation, opaque eyes, curved spine, hemorrhaging, molting, and cannibalism should be reported.

CHEMICAL AND PHYSICAL DATA

The dissolved oxygen concentration and pH must be measured at the beginning of the test, and every 24 hours thereafter, in the control and in the high, medium, and low effluent concentrations, for the duration of the test. The specific conductance, total alkalinity, total hardness, and salinity, where applicable, should be measured at the beginning of the test in the control and the high, medium and low effluent concentrations. There may be a build-up of ammonia in the static toxicity tests. It is advisable, therefore, to measure the concentration of total ammonia nitrogen in the control, high, medium, and low effluent concentrations at the beginning and end of each static test. The percentage of un-ionized ammonia in the test containers can be determined from Tables 5 and 6. Temperature should be recorded at least hourly in at least one container during the acclimation period and test.

TABLE 5. PERCENTAGE OF AMMONIA THAT IS UN-IONIZED IN DISTILLED WATER AT VARIOUS TEMPERATURES AND pH's[a]

PERCENT UN-IONIZED AMMONIA[b]									
Temperature	pH								
(°C)	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
7	0.01	0.05	0.15	0.46	1.45	4.44	12.8	31.7	59.5
12	0.02	0.07	0.22	0.68	2.13	6.44	17.9	40.8	68.5
17	0.03	0.10	0.32	1.00	3.08	9.14	24.1	50.1	76.1
22	0.04	0.14	0.45	1.43	4.39	12.7	31.5	59.2	82.1
27	0.06	0.21	0.65	2.03	6.15	17.2	39.6	67.4	86.8

TABLE 6. SALINITY CORRECTION FACTOR[c]

Salinity (g/kg)	Factor
5	0.82
10	0.80
15	0.77
20	0.75
25	0.73
30	0.72
35	0.72

[a] Skarheim (1973), and Thurston *et al.*(1974).

[b] These values can be corrected for salinity by multiplying by the appropriate factor from Table 6.

[c] The resulting values should be within about ±5% over the range of pH from 6 to 9, and temperature from 0° to 30°C.

Water samples collected for chemical analysis should be taken midway between the top, bottom, and sides of the test containers and should not include any surface scum or material stirred up from the bottom or sides.

Methods used for chemical analysis must be those specified in Section 304(g) of the Federal Water Pollution Control Act Amendments of 1972 (Chemical Methods Manual, USEPA, 1977).

CALCULATION OF LC50 AND EC50

For each set of data, the 96-hr LC50 or EC50 and its 95-percent confidence limits must be calculated on the basis of the initial volume percent of the effluent in the test solutions. The "volume percent" equals "(100 x volume of effluent)/(volume of effluent + volume of dilution water)." If other (24-,48-,72-hr) LC and EC values are calculated, their 95 percent confidence limits must also be determined. A variety of methods are available to calculate a LC and EC (Finney, 1964, 1971). The most widely used are the log-concentration versus-percent-survival, probit, logit, moving-average, and Litchfield-Wilcoxon (1949) methods.

Two examples of calculating a LC50 using a hypothetical set of data have been provided in the Appendix. The two methods used in the calculations are: (1) the Litchfield-Wilcoxon and, (2) the log-concentration-versus-percent-survival. If more than 10 percent of the control organisms die, none of the previously mentioned methods may be used in calculating LC and EC values, and the remaining test results must be used with caution in evaluating the toxic effect.

REPORTS

A report of the results of a test must include the following:

A. The name of the test method, investigator and laboratory, and the date the test was conducted.

B. A detailed description of the effluent, including its source, date and time of collection, composition, known physical and chemical properties, and variability.

C. The source of the dilution water, the date and time of its collection, its chemical characteristics, and a description of any pretreatment.

D. Detailed information about the test organisms, including scientific name, length and weight, age, life stage, source, history, observed diseases, treatments, and acclimation procedure used.

E. A description of the test procedure: the test chambers, including the depth and volume of solution; the way the test was begun; the number of organisms per treatment; and the loading. For the flow-through system, the water volume changes per 24 hours in each test chamber must be calculated and reported.

F. The definition of the adverse effect (death, immobility, etc.) used in the test, and a summary of general observations on other effects or symptoms.

G. The number and percentage of organisms in each test chamber (including the control chambers) that died or showed the "effect" used to measure the toxicity of the effluent.

H. A 24-, 48-, 72-, and 96-hr LC50 or EC50 value for the test organisms, depending on the duration of exposure. If 100 percent effluent did not kill or affect more than 65 percent of the test organisms, report the percentage of the test organisms killed or affected by various concentrations of the effluent.

I. The 95-percent confidence limits for the LC50 and EC50 values and the method used to calculate them.

J. The methods used for and the results of all chemical analyses.

K. The average and range of the acclimation temperature and the test temperature.

L. Any deviation from this method.

M. Any other relevant information.

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APPENDIX

A. LITCHFIELD AND WILCOXON ABBREVIATED METHOD OF DETERMINING THE LC50

General Procedure

Step 1: Tabulate the data (see sample data sheet, Fig. 1, p. 33) showing the percent-effluent volumes used, the total number of organisms exposed to each percent-effluent volume, the number of affected organisms, and the observed percent-affected organisms (see Example 1 below). Do not list more than 2 consecutive 100 percent affects at the higher percent-effluent volumes or more than two consecutive 0 percent affects at the lower percent-effluent volumes.

Step 2: Plot the percent-affected organisms against the percent-effluent volume on 2 cycle, logarithmic probability paper (Fig. 2), except for 0 percent or 100 percent affect values. With a straight edge, fit a temporary line through the points, particularly those in the region of 40 percent to 60 percent affects.

Step 3: Using the line drawn through the points, read and list an "expected" percent affect for each percent-effluent volume tested. Disregard the "expected" percent value for any of the percent volumes less than 0.01 or greater than 99.99. Using the expected-percent-affect, calculate from Table 7 a "corrected" value for each 0 percent or 100 percent affect obtained in the test. (Since the expected values in the table are whole numbers, it will be necessary to obtain intermediate values by interpolation.) Plot these values on the logarithmic probability paper (Fig. 2) used in Step 2 and inspect the fit of the line to the completely plotted data. If after plotting the corrected expected values for 0 percent and 100 percent affected, the fit is obviously unsatisfactory, redraw the line and obtain a new set of expected values.

Step 4: List the difference between each observed (or corrected) value and the corresponding expected value. Using each difference and the corresponding expected value, read and list the contributions to Chi-square (Chi^2) from Fig. 3 (a straight edge connecting a value on the Expected-Percent Affected scale with a value on the Observed-Minus-Expected scale, will indicate at the point of intersection of the Chi^2 scale, the contribution to Chi^2. Sum the contributions to Chi^2 and multiply the total by the average number of organisms per effluent volume, i.e., the number of organisms used in K concentrations divided by K, where K is the number of percent-affected organism values plotted. The product is the "calculated" Chi^2 of the line. The degrees of freedom (N) are 2 less than the number of points plotted, i.e., $N = K-2$. If the calculated Chi^2 is less than the Chi^2 given in Table 8 for N degrees of freedom, the data are non-heterogeneous and the line is a good fit. However, if the calculated Chi^2 is greater than the Chi^2 given in Table 8 for N degrees of freedom, the data are heterogeneous and the line is not a good fit. In the event a line cannot be fitted (the calculated Chi^2 is greater than the tabular Chi^2), the data can not be used to calculate a LC50 or EC50. Litchfield and Wilcoxon provided an alternate method for calculating the 95 percent confidence limits under these circumstances. However, the toxicity test should be repeated.

Step 5: Determine the confidence limits of the LC50.

a. Read from the fitted line (Fig. 2), the percent effluent volumes for the corresponding 16, 50, 84 percent affects (LC16, LC50 and LC84).

b. Calculate the slope function, S, as:

$$S = \frac{LC84/LC50 + LC50/LC16}{2}$$

c. From the tabulation of the data determine N', which is defined as the total number of test organisms used within the percent-affected-organism interval of 16 percent and 84 percent. Calculate the exponent $(2.77/\sqrt{N'})$ for the slope function and the factor, f_{LC50}, used to establish the confidence limits for the LC50 (or EC50).

$$f_{LC50} = S^{(2.77/\sqrt{N'})}$$

The f_{LC50} can be obtained directly from the nomogram in Fig.4 by laying a straight-edge across the appropriate base and exponent values and reading the resultant "f" value.

e. Calculate the confidence limits of the LC50 as follows:

(1) Upper limit for 95% probability = LC50 X f_{LC50}

(2) Lower limit for 95% probability = LC50/f_{LC50}

Example

Steps 1-4: The data were tabulated and plotted (Fig. 2) and the expected values were read from the graph.

STEP ONE				STEP THREE	STEP FOUR	
% Effluent Volume	Number of Organisms	Number of Affected Organisms	Observed % Affected Organisms	Expected % (Fig. 2)	Observed Minus Expected	Chi^2
3.2	20	0	0(.2)[b]	.6	0.4	0.003
5.6	20	1	5	3.5	1.5	0.006
10.0	20	11	55	(14.5)[a]	Aberrant Value	
18.0	20	7	35	38.0	3.0	0.004
32.0	20	12	60	67.0	7.0.	0.024
56.0	20	18	90	87.5	2.5	0.006
100.0	20	20	100 (99.0)[b]	97.0	2.0	0.014
					Total	0.057

a. Percent-affected organisms at the 10 percent effluent volume is obviously an aberrant value and should be omitted when fitting the line in Step 2.

b. Step 3 "Corrected" affected values from Table 7.

Step 4 (Cont.):

Calculation of Chi^2

a. Mean number of organisms used in 'K' (K=6) concentrations = $\frac{120}{6}$ = 20.

(Note that the data for the 10% effluent volume were aberrent and not used. Therefore, K=6, and the total number of organisms = 120)

b. Calculated $\text{Chi}^2 = 20 \times 0.057 = 1.14$

c. Degrees of Freedom (N) = K - 2 = 6 - 2 = 4

d. From Table 8, the Chi^2 for 4 degrees of freedom = 9.49

e. The calculated Chi^2 is less than the tabular Chi^2. Therefore, it is assumed the line is a good fit, and the data are non-heterogeneous.

Step 5:

a. From the fitted line in Fig. 2, determine the (percent) effluent concentrations corresponding to the 16%, 50% and 84% affected organism values:

b. LC84 effluent concentrations = 50.0%
LC50 " " = 23.0%
LC16 " " 10.5%

c. Calculate the slope function, 'S', as:

$$S = \frac{LC84/LC50 + LC50/LC16}{2} = \frac{50.0/23.0 + 23.0/10.5}{2}$$

$$= \frac{2.17 + 2.19}{2} = \frac{4.36}{2} = 2.18$$

d. N' = 40 (From Figure 2)

e. Calculate the exponent (N') and factor, f_{LC50}

$$f_{LC50} = S^{2.77/\sqrt{N'}} = 2.18^{2.77/\sqrt{40}} = 2.18^{2.77/6.32} = 2.18^{0.438} = 1.41$$

f. Calculate the confidence limits of the LC50

(1) Upper limit for 95% probability = LC50 X f_{LC50} = 23.0 X 1.4 = 32.2%

(2) Lower limit for 95% probability = LC50/f_{LC50} = 23.0/1.4 = 16.4%

TABLE 7. CORRECTED VALUES OF 0% OR 100% EFFECT

Expected Value	Corrected Value									
	0	1	2	3	4	5	6	7	8	9
0	--	0.3	0.7	1.0	1.3	1.6	2.0	2.3	2.6	2.9
10	3.2	3.5	3.8	4.1	4.4	4.7	4.9	5.2	5.5	5.7
20	6.0	6.2	6.5	6.7	7.0	7.2	7.4	7.0	7.8	8.1
30	8.3	8.4	8.6	8.8	9.0	9.2	9.3	9.4	9.6	9.8
40	9.9	10.0	10.1	10.2	10.3	10.4	10.4	10.4	10.4	10.5
50	--	89.5	89.6	89.6	89.6	89.7	89.7	89.8	89.9	90.0
60	90.1	90.2	90.4	90.5	90.7	90.8	91.0	91.2	91.4	91.6
70	91.7	91.9	92.2	92.4	92.6	92.8	93.0	93.3	93.5	93.8
80	94.0	94.3	94.5	94.8	95.1	95.3	95.6	95.9	96.2	96.5
90	96.8	97.1	97.4	97.7	98.0	98.4	98.7	99.0	99.3	99.7

TABLE 8. VALUES OF Chi^2 ($p = 0.05$)

Degrees of Freedom (N)	Chi^2
1	3.84
2	5.99
3	7.82
4	9.49
5	11.1
6	12.6
7	14.1
8	15.5
9	16.9
10	18.8

FIG. I. DATA SHEET FOR EFFLUENT TOXICITY TEST.

Industry/Toxicant ____________________
Address ____________________
Contact ____________________
Effluent Serial Number ____________________
NPDES Permit Number ____________________

Beginning: Date ________ Time ________
Ending: Date ________ Time ________
Test Organism ____________________
Test Temperature Range ____________________

Conc. or %	Test Container Number	Number of Live Organisms					Dissolved Oxygen (mg/l)					pH					Total Alkalinity (mg/l as $CaCO_3$)					Total Hardness (mg/l as $CaCO_3$)									
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96

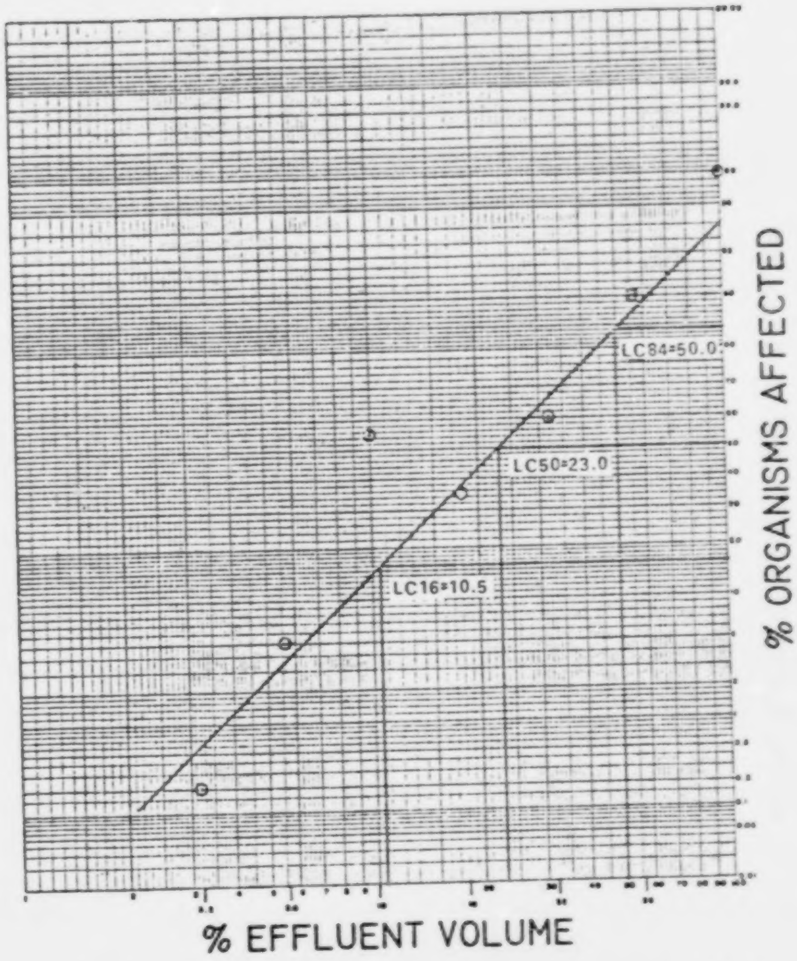


FIG. 2. LINE FITTED TO DATA, AND LC16, LC50, AND LC84 AS READ FROM THE LINE (STEPS 2, 3, AND 5).

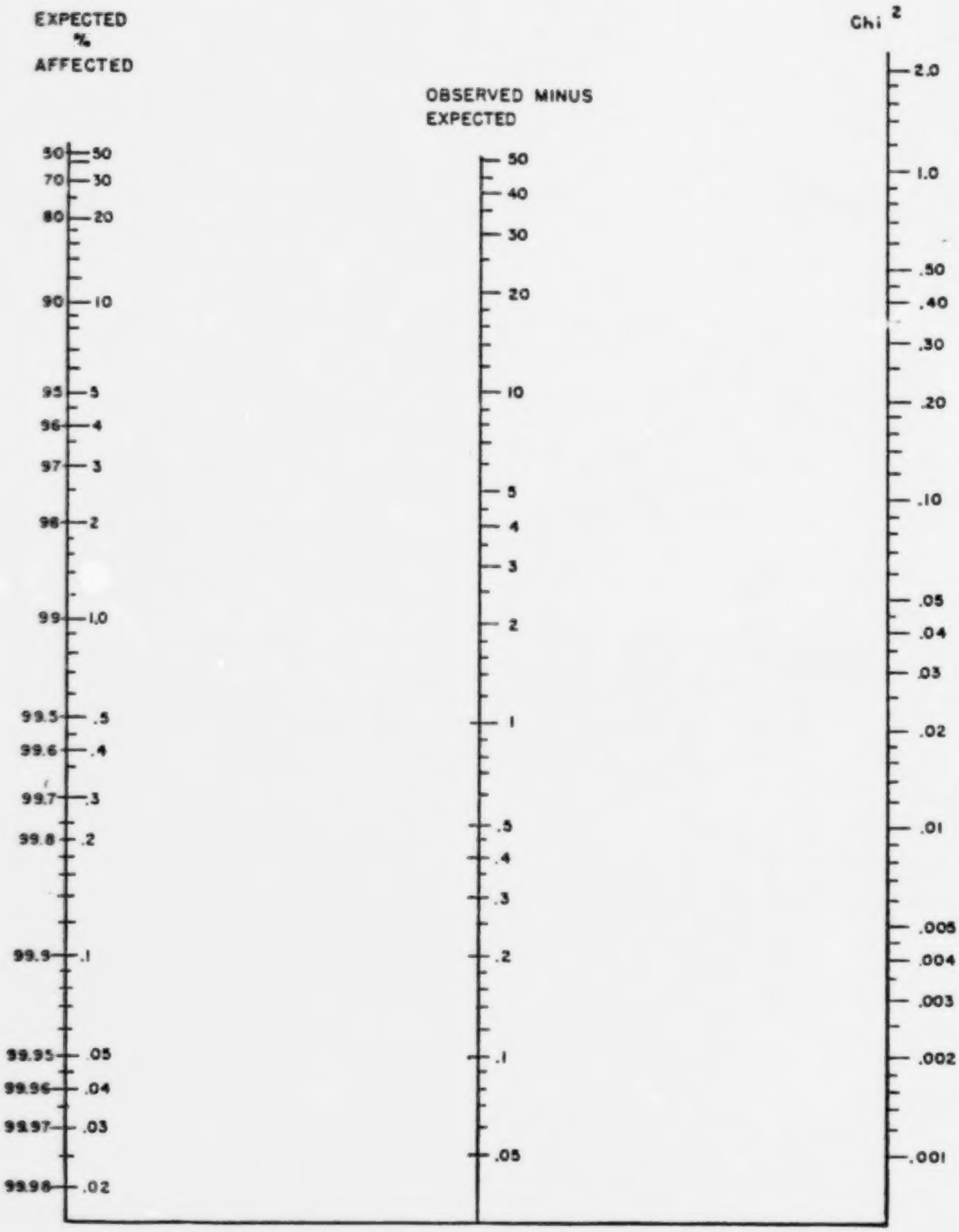


FIG. 3. NOMOGRAPH FOR OBTAINING Chi^2 FROM EXPECTED % AFFECTED AND OBSERVED–MINUS–EXPECTED (STEP 4).

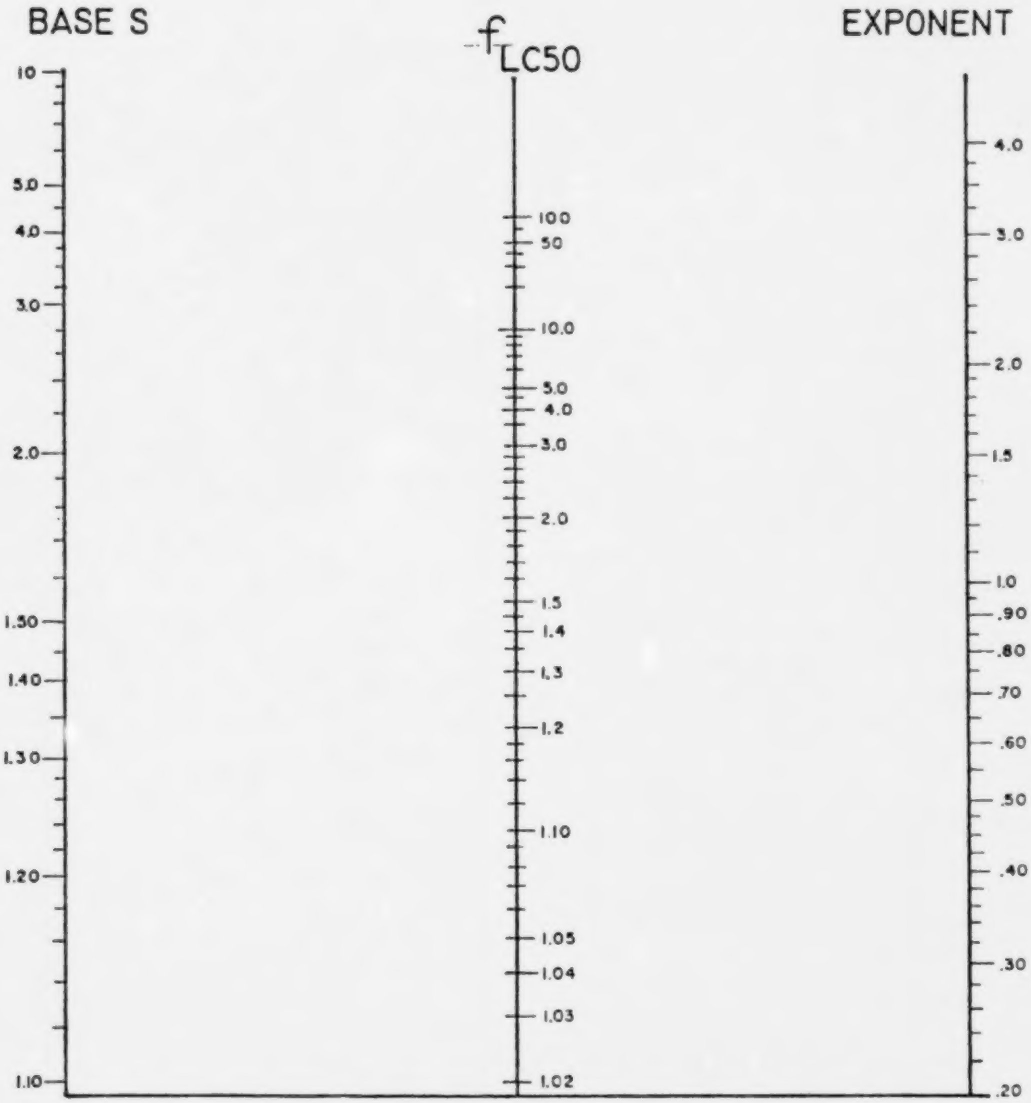


FIG. 4. NOMOGRAPH FOR RAISING BASE S TO A FRACTIONAL EXPONENT

B. LOG-CONCENTRATION VERSUS PERCENT-SURVIVAL METHOD OF DETERMINING THE LC50

General Procedure

Step 1: Plot the percent effluent volumes and the corresponding percent survival on semi-logarithmic paper (Fig. 5).

Step 2: Locate the 2 highest points on the graph which are separated by the 50 percent survival line and connect them with a diagonal straight line. However, if one of the points is an aberrant value, the next lowest or highest percent-effluent volume is used.

Step 3: Read on the scale for percent-effluent volume, the value of the point where the diagonal line and the 50 percent survival line intersect. This value is the LC50 percent-effluent volume for the test. If by chance one of the effluent concentrations happens to have 50 percent survival, no graphing is necessary.

Example

Step 1: The percent-effluent volumes and the corresponding percent survival data from the Litchfield and Wilcoxon example are plotted in Fig. 5.

Step 2: The two highest points which are separated by the 50 percent survival line (65 percent and 40 percent) are located and connected with a diagonal diagonal straight line. The percent survival in the 10 percent-effluent volume was considered an aberrant value and, therefore, was omitted from the evaluation.

Step 3: An LC50 of 25.4 percent-effluent volume for the test was derived from the point where the diagonal line and the 50 percent survival line intersected in Fig.5.

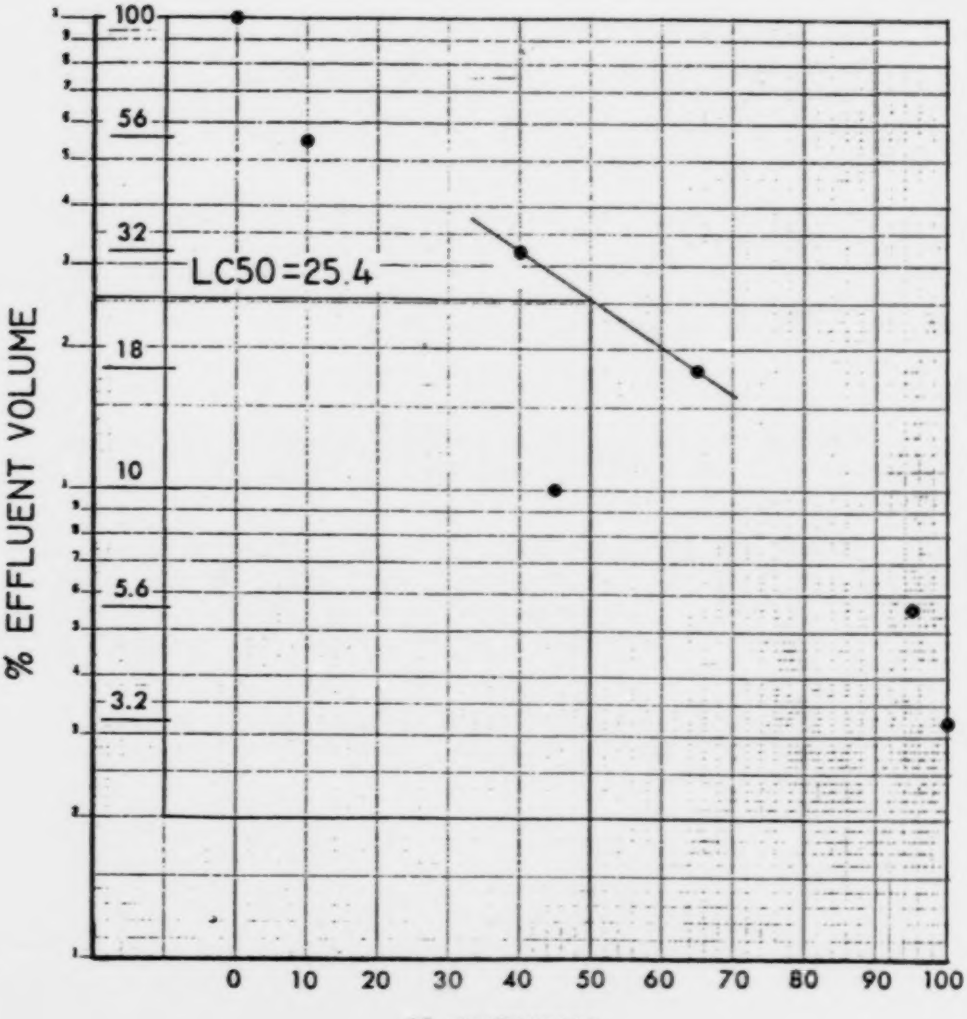


FIG. 5. PLOTTED DATA AND FITTED LINE FOR LOG—CONCENTRATION VERSUS % SURVIVAL METHOD

C. DILUTOR SYSTEMS, CONTROL PANEL AND EQUIPMENT LISTS

Two proportional dilutor systems are illustrated: (1) The Solenoid Valve System, and (2) the Vacuum Siphon System. The designs incorporate features from devices developed by many other Federal and State programs, and have been shown to be very versatile for on-site bioassays in mobile labs as well as in fixed (central) labs. The Solenoid Valve System is fully controlled by solenoids (Figs. 6, 7, & 8), and is the preferred system. The Vacuum Siphon System (Figs. 6, 9, & 10), however is acceptable. Both systems employ the same control panel (Fig. 14).

If in the range-finding test, the LC50 of the effluent falls in the concentration range, 5.6% to 100%, premixing is not required, and the mixing chamber is by-passed by running a TEFLON^R tube directly from the effluent in-flow pipe to chamber E-1. Chambers D-1 and D-2 and the mixing chamber are deactivated.

To provide the capability of using the dilutor system to carry out tests of the toxicity of pure compounds, the control panel is equipped with an auxillary power receptacle to operate a metering pump to deliver an aliquot of the stock solution of the pure compound directly to the mixing chamber during each cycle. In this case, chamber D-1 is deactivated and chamber D-2 is calibrated to deliver a volume of 2000 ml, which is used to dilute the aliquot to the highest concentration used in the toxicity test.

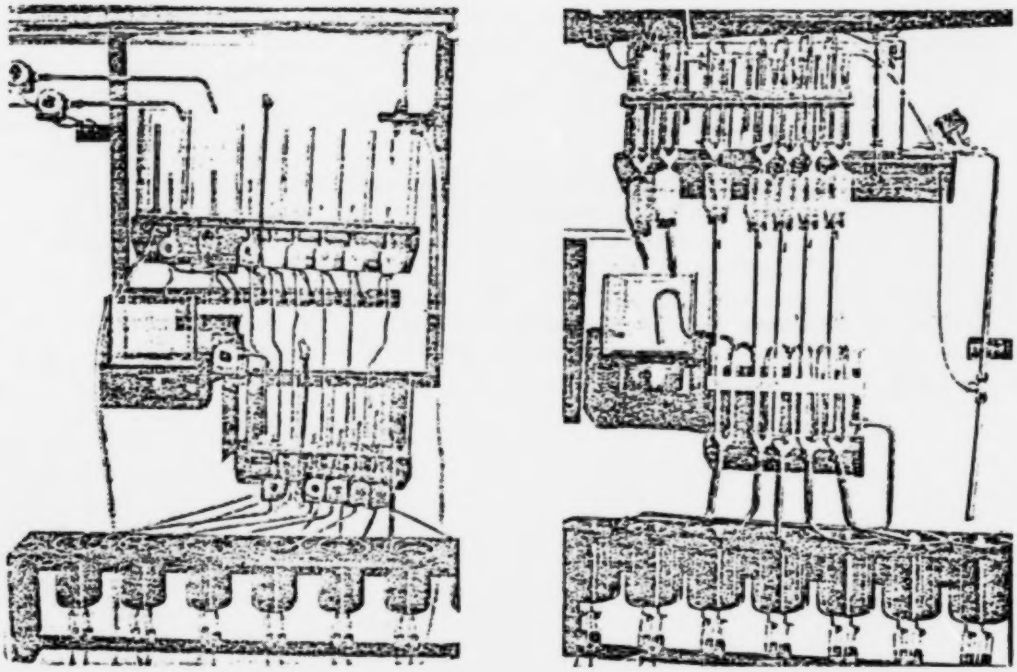


FIG. 6. PHOTOGRAPHS OF DILUTOR SYSTEMS: SOLENOID VALVE SYSTEM (LEFT), AND VACUUM SIPHON SYSTEM (RIGHT).

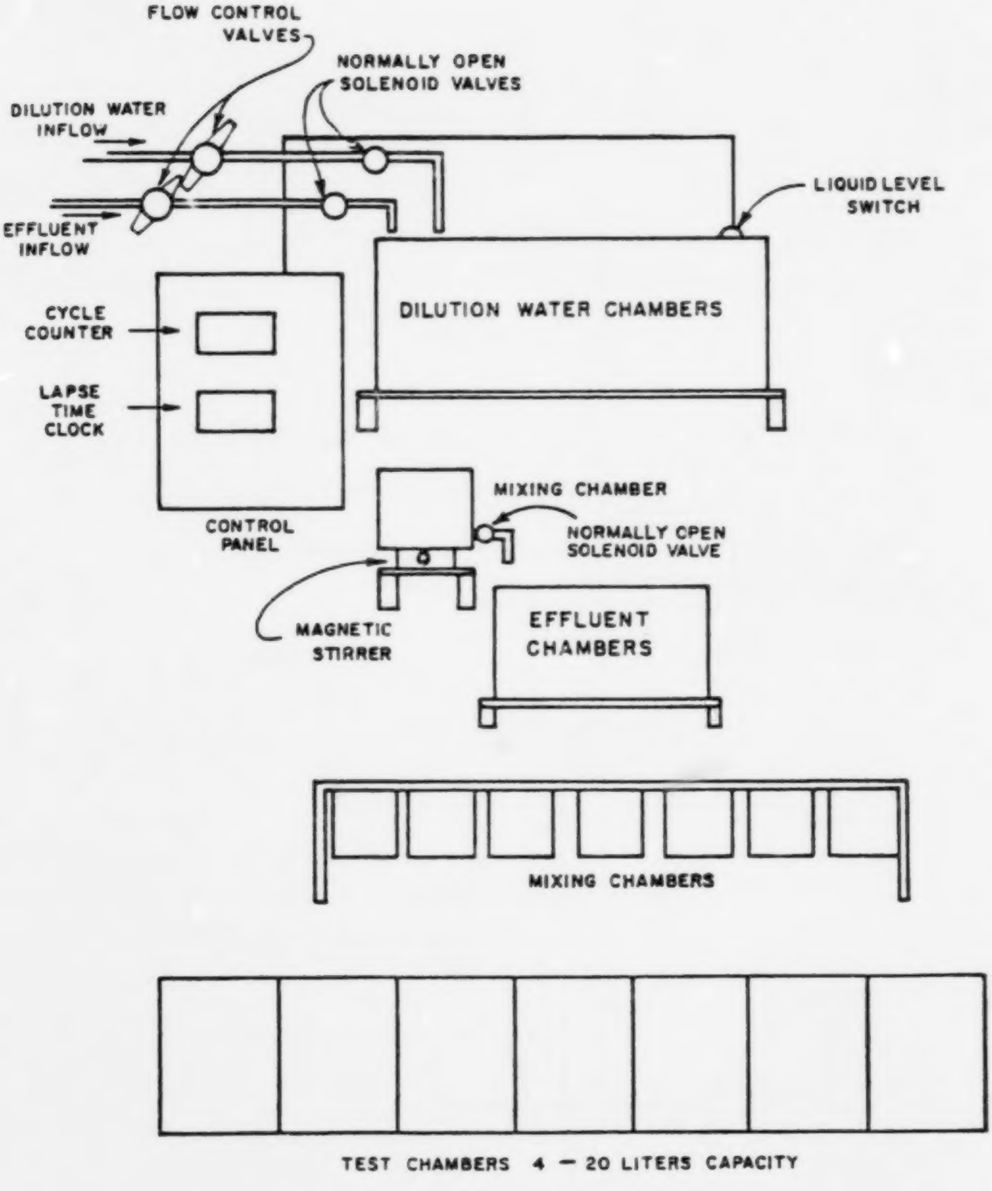

FLOW CONTROL VALVES
NORMALLY OPEN SOLENOID VALVES
DILUTION WATER INFLOW
EFFLUENT INFLOW
LIQUID LEVEL SWITCH
DILUTION WATER CHAMBERS
CYCLE COUNTER
LAPSE TIME CLOCK
CONTROL PANEL
MIXING CHAMBER
NORMALLY OPEN SOLENOID VALVE
MAGNETIC STIRRER
EFFLUENT CHAMBERS
MIXING CHAMBERS
TEST CHAMBERS 4 — 20 LITERS CAPACITY

FIG. 7. SOLENOID VALVE DILUTOR SYSTEM, GENERAL DIAGRAM.

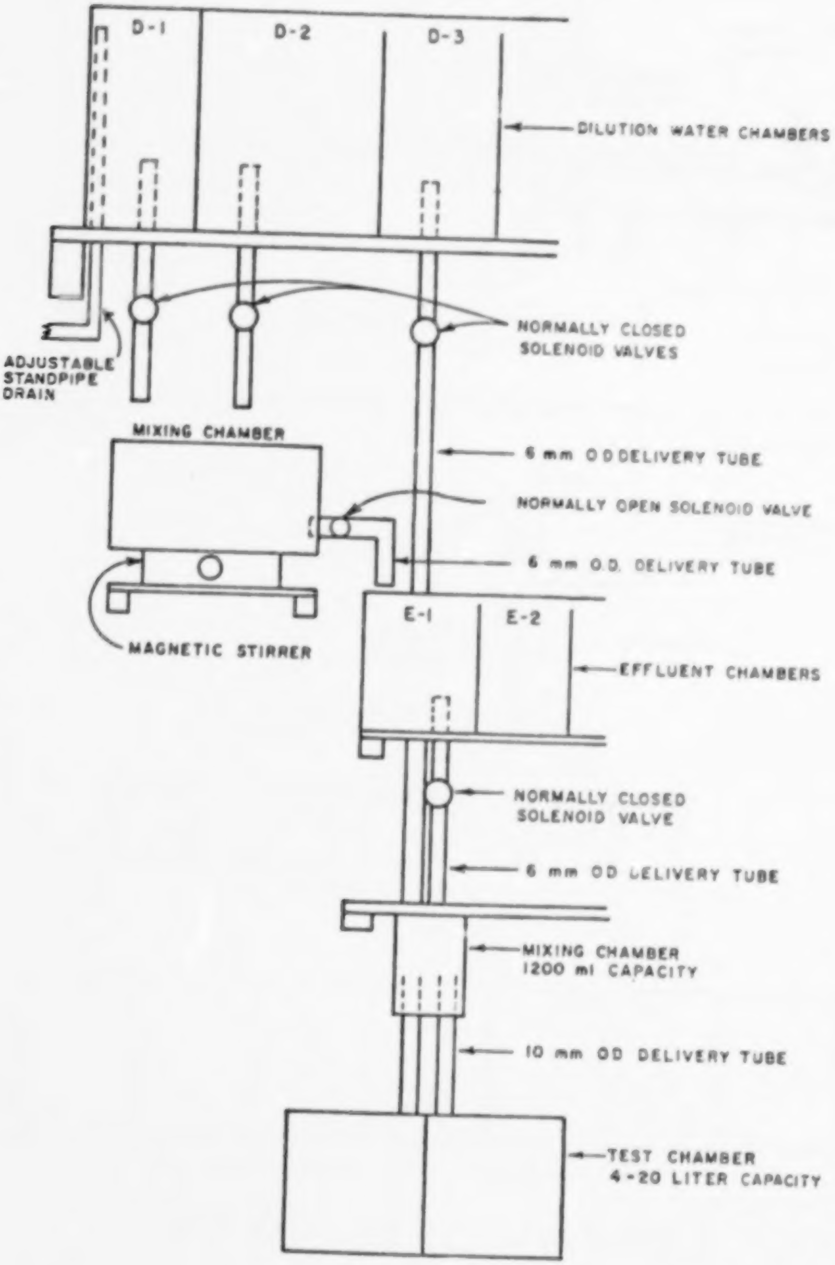

D-1
D-2
D-3
DILUTION WATER CHAMBERS
NORMALLY CLOSED SOLENOID VALVES
ADJUSTABLE STANDPIPE DRAIN
MIXING CHAMBER
6 mm O.D DELIVERY TUBE
NORMALLY OPEN SOLENOID VALVE
6 mm O.D. DELIVERY TUBE
MAGNETIC STIRRER
E-1
E-2
EFFLUENT CHAMBERS
NORMALLY CLOSED SOLENOID VALVE
6 mm OD DELIVERY TUBE
MIXING CHAMBER 1200 ml CAPACITY
10 mm OD DELIVERY TUBE
TEST CHAMBER 4-20 LITER CAPACITY

FIG. 8. SOLENOID VALVE DILUTOR SYSTEM, DETAILED DIAGRAM.

Solenoid System Equipment List

1. Diluter Glass.
2. Stainless Steel Solenoid Valves
   a. 3 - normally open, two-way, 55 psi, water, 1/4" pipe size, 9/32" orifice size, ASCO 8262152, for incoming effluent and dilution water pipes and mixing chamber pipe.
   b. 1 - normally closed, two-way, 15 psi, water, 3/8" pipe size, 3/8" orifice size, ASCO 8030B65, for D-2 chamber evacuation pipe.
   c. 12 - normally closed, two-way, 36 psi, water, 1/4" pipe size, 9/32" orifice size. ASCO 8262C38, for remaining dilution chamber and effluent chamber evacuation pipes.
3. Stainless steel tubing, seamless, 316-grade, austenetic.
   a. 10 ft - 3/8" OD, 0.035" wall thickness, for dilution water and effluent pipes.
   b. 60 ft - 1/4" OD, 0.035" wall thickness, for dilution water and effluent pipes.
   c. 1 ft - 3/4" OD, 0.035" wall thickness, for standpipe in D-1 chamber.
4. Swagelok tube connectors, stainless steel.
   a. 4 - male tube connectors, male pipe size 1/4", tube OD 3/8".
   b. 2 - male tube connectors, male pipe size 1/2", tube OD 3/8".
   c. 26 - male tube connectors, male pipe size 1/4, tube OD 1/4"
   d. 2 - male tube connectors, male pipe size 3/8", tube OD 3/8".
   e. 2 - male adapter, tube to pipe, male size 1/2", tube OD 3/8".
5. 7 - 1200 ml stainless steel beakers.
6. Several lbs each of Neoprene stoppers, size 00, 0, and 1, 1 lb of siz 5.
7. 14 - aquarium 2-5 gal.
8. Magnetic stirrer.
9. 2 - PVC ball valves, 1/2" pipe size.
10. Dilutor control panel - see Fig. 14 and equipment list.
11. Plywood sheeting, exterior grade: one - 4' x 8' x 3/4", one - 4' x 8' x 1/2".
12. Pine or redwood board, 1" x 8", 20 ft.
13. Epoxy paint, 1 gal.
14. Assorted wood screws, nails, etc.
15. 25 ft - 1/4" ID, TEFLON^{R} tubing, to connect the mixing chambers to the test chambers (see Fig. 10).

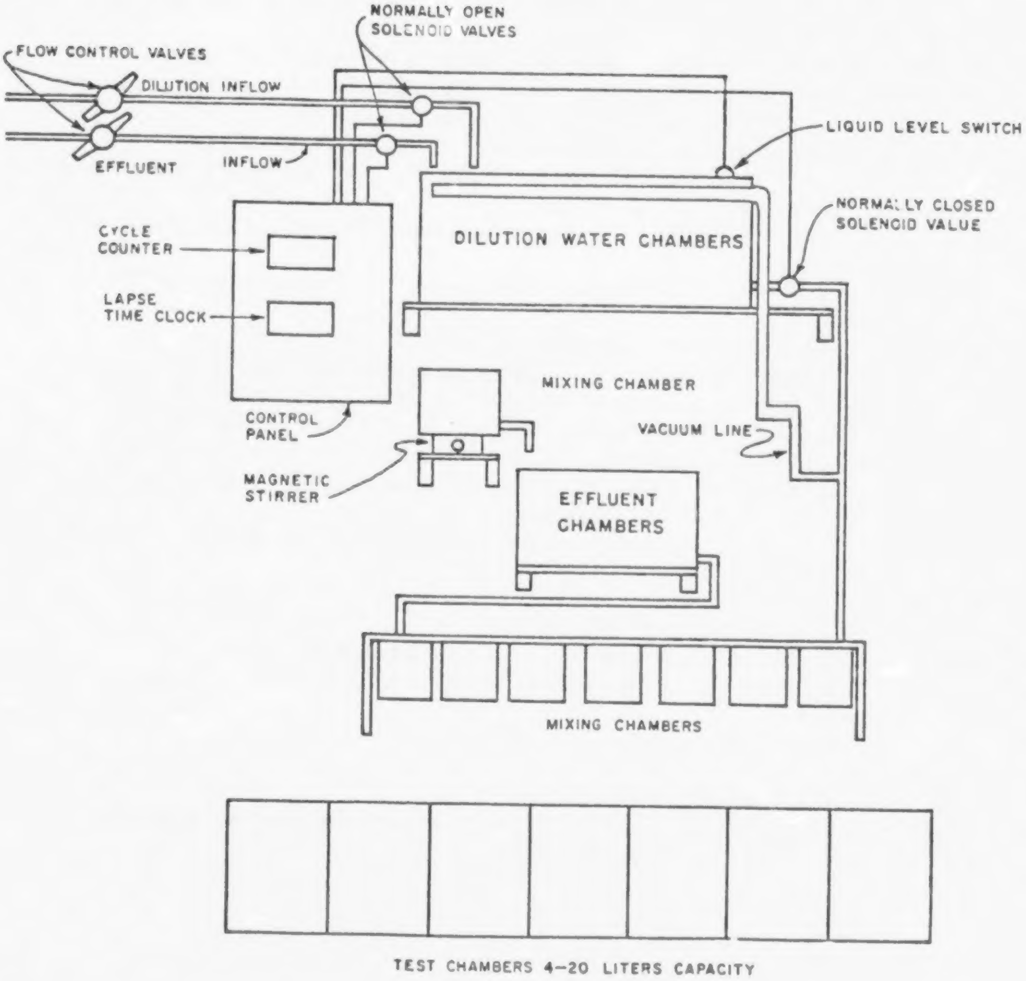

NORMALLY OPEN SOLENOID VALVES
FLOW CONTROL VALVES
DILUTION INFLOW
EFFLUENT
INFLOW
LIQUID LEVEL SWITCH
NORMALLY CLOSED SOLENOID VALUE
CYCLE COUNTER
DILUTION WATER CHAMBERS
LAPSE TIME CLOCK
MIXING CHAMBER
CONTROL PANEL
VACUUM LINE
MAGNETIC STIRRER
EFFLUENT CHAMBERS
MIXING CHAMBERS
TEST CHAMBERS 4–20 LITERS CAPACITY

FIG. 9. VACUUM SIPHON DILUTOR SYSTEM, GENERAL DIAGRAM.

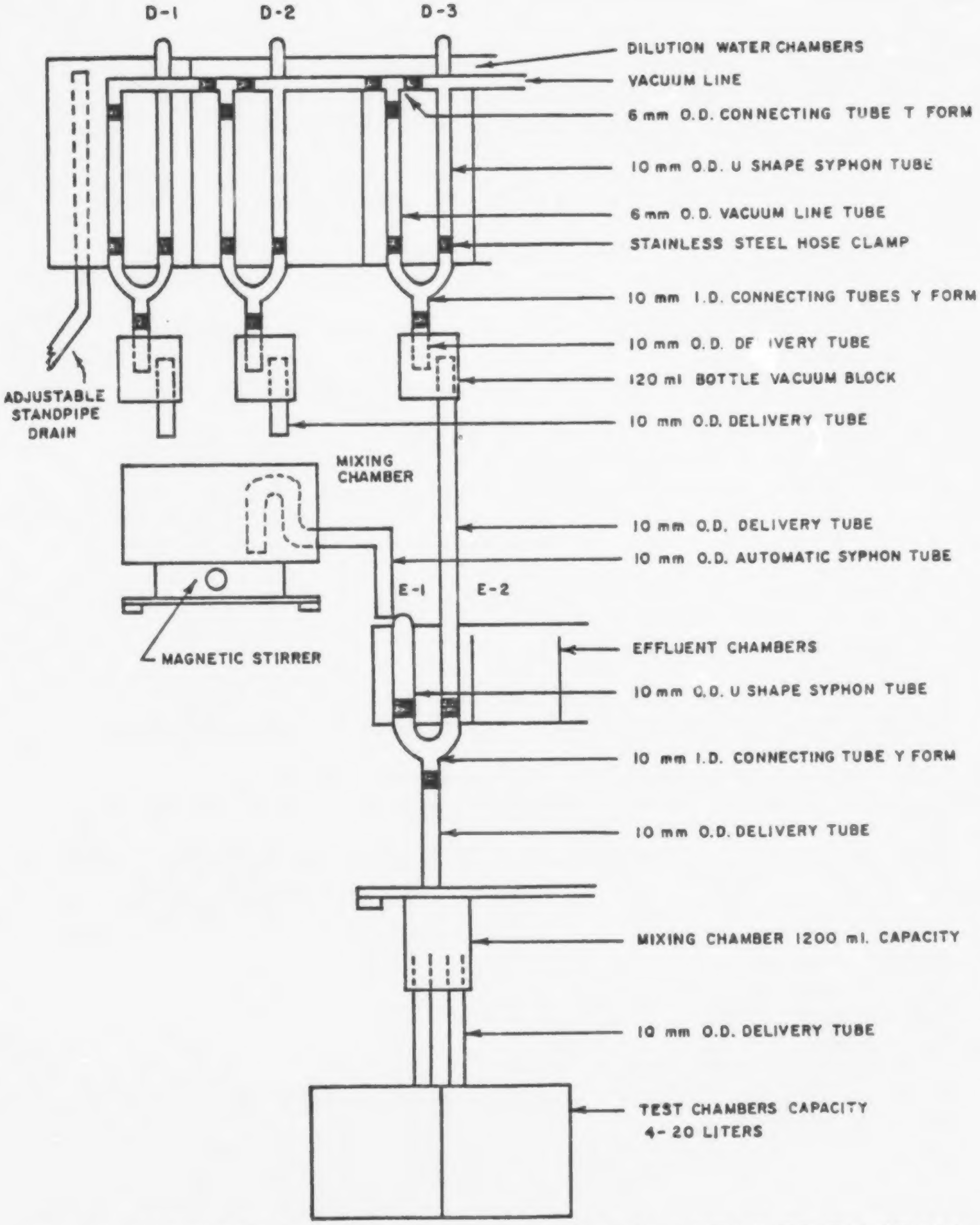
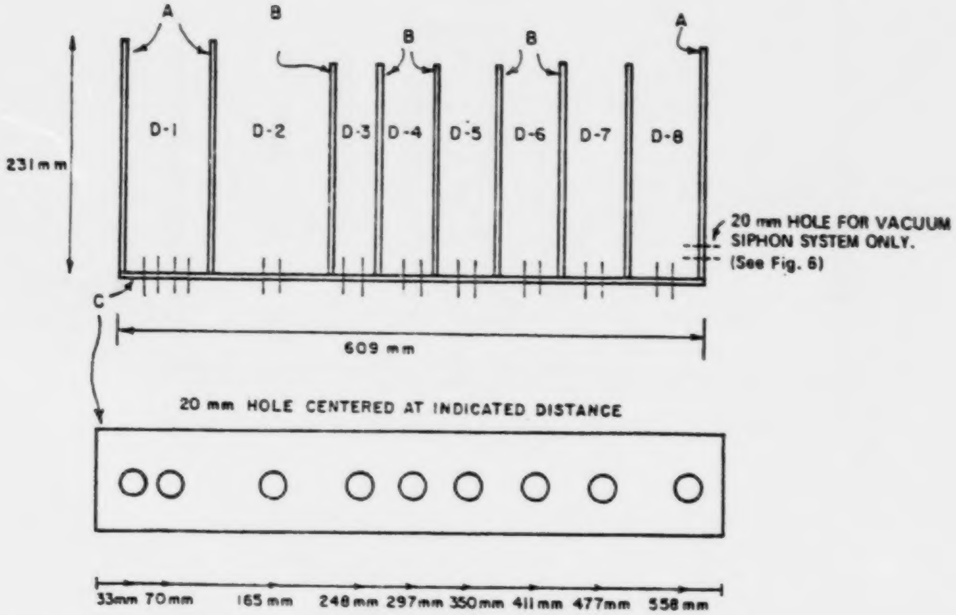

D-1
D-2
D-3
DILUTION WATER CHAMBERS
VACUUM LINE
6 mm O.D. CONNECTING TUBE T FORM
10 mm O.D. U SHAPE SYPHON TUBE
6 mm O.D. VACUUM LINE TUBE
STAINLESS STEEL HOSE CLAMP
10 mm I.D. CONNECTING TUBES Y FORM
10 mm O.D. DELIVERY TUBE
120 ml BOTTLE VACUUM BLOCK
10 mm O.D. DELIVERY TUBE
ADJUSTABLE STANDPIPE DRAIN
MIXING CHAMBER
10 mm O.D. DELIVERY TUBE
10 mm O.D. AUTOMATIC SYPHON TUBE
E-1
E-2
MAGNETIC STIRRER
EFFLUENT CHAMBERS
10 mm O.D. U SHAPE SYPHON TUBE
10 mm I.D. CONNECTING TUBE Y FORM
10 mm O.D. DELIVERY TUBE
MIXING CHAMBER 1200 ml. CAPACITY
10 mm O.D. DELIVERY TUBE
TEST CHAMBERS CAPACITY
4-20 LITERS

FIG. 10. VACUUM SIPHON DILUTOR SYSTEM, DETAILED DIAGRAM.

Vacuum Siphon System Equipment List

1. Diluter Glass.
2. Stainless steel solenoid valves.
    a. 2 - normally open, two-way, 55 psi, water, 1/4" pipe size, 9/32" orifice size, ASCO 8262152, for incoming effluent and dilution water pipes.
    b. 1 - normally closed, two-way, 15 psi, water, 3/8" pipe size, 3/8" orifice size, ASCO 8030B65, for dilution water evacuation pipe.
3. Stainless steel tubing, seamless, 316-grade, austenetic.
    a. 60 ft - 3/8" OD, 0.035" wall thickness, for dilution water and effluent pipes.
    b. 20 ft - 5/16" OD, 0.035" wall thickness. for standpipes in mixing chambers.
    c. 1 ft - 3/4" OD, 0.035" wall thickness, for standpipe in D-1 chamber.
4. Swagelok tube connectors, stainless steel
    a. 4 - male tube connectors, male pipe size 1/4", tube OD 3/8".
    b. 2 - male tube connectors, male pipe size 3/8", tube OD 3/8".
    c. 2 - male adapter, tube to pipe, male pipe size 1/2", tube OD 3/8".
    d. 2 - male tube connectors, male pipe size 1/2", tube OD 3/8".
5. 7 - 1,200 ml stainless steel beakers.
6. Several lbs each of NEOPRENE^R stoppers, size 00, 0 and 1; 1 lb of size 5.
7. 14 - aquariums, 2-5 gal.
8. Magnetic stirrer.
9. 2 - PVC Ball valves, 1/2" pipe size.
10. Dilutor control panel equipment - see Fig. 14 and equipment list.
11. 7 - 120 ml NALGENE^R bottles.
12. 3 ft, 1-in-2 aluminum bar, for siphon support brackets.
13. Stainless steel set screws, box of 50, for securing SS tubing in siphon support brackets.
14. Stainless steel hose clamps, box of 10, size #4 or 5,(need 3 boxes).
15. 6 - NALGENE^R T's, 5/16" OD.
16. 12 - TYGON^R Y connectors, 3/8" I.D.
17. TYGON^R tubing, 3/8" OD, 10 ft.
18. Plywood sheeting, exterior grade: one - 4' x 8x x 3/4", one - 4' x 8' x 1/2".
19. Pine or redwood board, 1" x 8", 20 ft.
20. Epoxy paint, 1 gal.
21. Assorted wood screws, nails, etc.
22. 25 ft - 5/16" ID, TEFLON^R tubing, to connect the mixing chambers to the test chambers.


A
B
B
B
A
231mm
D-1
D-2
D-3
D-4
D-5
D-6
D-7
D-8
20 mm HOLE FOR VACUUM SIPHON SYSTEM ONLY. (See Fig. 6)
C
609 mm
20 mm HOLE CENTERED AT INDICATED DISTANCE
33mm 70mm 165 mm 248mm 297mm 350mm 411mm 477mm 558mm

DRAIN HOLES IN BOTTOM PLATE (C) SHOWN FOR SOLENOID VALVE DILUTOR SYSTEM. FOR VACUUM SIPHON DILUTOR SYSTEM, SINGLE DRAIN HOLE IS REQUIRED ONLY FOR CHAMBER D-1.

INDIVIDUAL PART SIZE AND NUMBER OF
PIECES USING 6 mm (1/4") PLATE GLASS

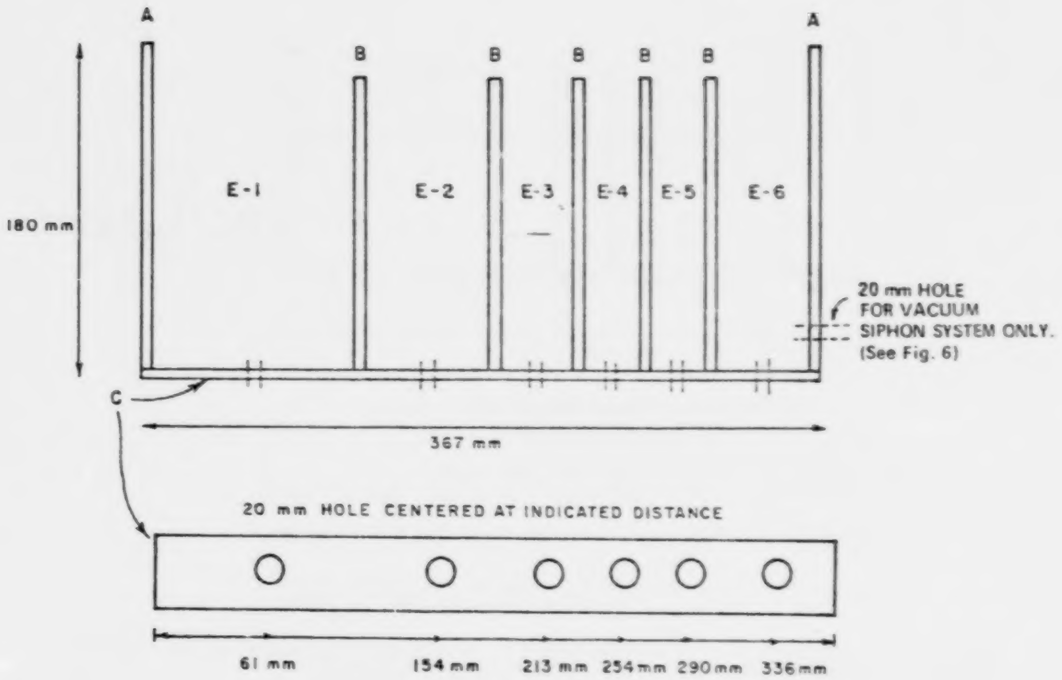
A 225 mm X 95 mm - 3
B 200 mm X 95 mm - 6
C 609 mm X 95 mm - 1 (Bottom Plate)
D 609 mm X 231 mm - 2 (Side Panels)

INSIDE CELL MEASUREMENTS AND APPROXIMATE VOLUME

D - 1 95 mm X 225 mm X 95 mm - 2030 ml
D - 2 115 mm X 200 mm X 95 mm - 2185 ml
D - 3 40 mm X 200 mm X 95 mm - 760 ml
D - 4 45 mm X 200 mm X 95 mm - 855 ml
D - 5 50 mm X 200 mm X 95 mm - 950 ml
D - 6 60 mm X 200 mm X 95 mm - 1140 ml
D - 7 60 mm X 200 mm X 95 mm - 1140 ml
D - 8 90 mm X 200 mm X 95 mm - 1710 ml

NOTE: 1/8" - 316 GRADE AUSTENISTIC STAINLESS STEEL
MAY BE SUBSTITUTED FOR GLASS IN PART C.

FIG. II. DILUTION WATER CHAMBERS


A
B
B
B
B
B
A
E-1
E-2
E-3
E-4
E-5
E-6
180 mm
20 mm HOLE
FOR VACUUM
SIPHON SYSTEM ONLY.
(See Fig. 6)
C
367 mm
20 mm HOLE CENTERED AT INDICATED DISTANCE
61 mm
154 mm
213 mm
254mm
290mm
336mm

DRAIN HOLES IN BOTTOM PLATE (C) SHOWN FOR SOLENOID VALVE DILUTOR SYSTEM. FOR VACUUM SIPHON DILUTOR SYSTEM, SINGLE DRAIN HOLE IS REQUIRED ONLY FOR CHAMBER D-1.

INDIVIDUAL PART SIZE AND NUMBER OF PIECES USING 6mm (1/4") PLATE GLASS

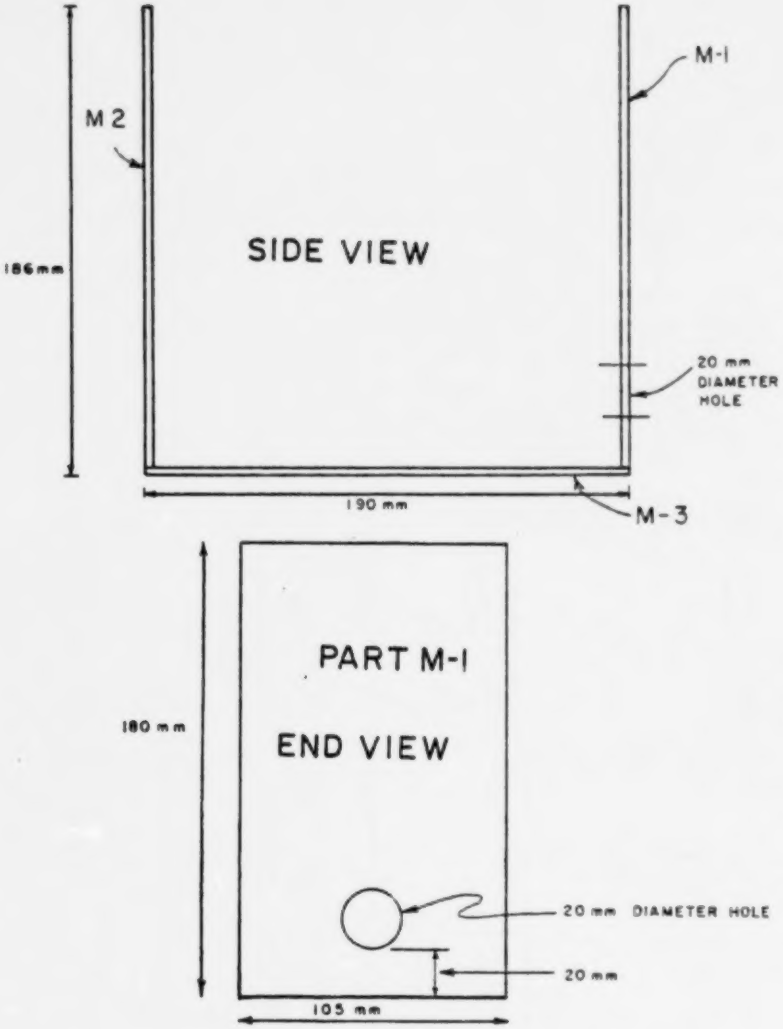
A 174 mm X 40 mm - 2
B 155 mm X 40 mm - 5
C 367mm X 40 mm - 1 (Bottom Plate)
D 367mm X 180 mm - 2 (Side Panels)

INSIDE CELL MEASUREMENTS AND APPROXIMATE VOLUME

E-1 110mm X 155mm X 40mm - 682 ml
E-2 65mm X 155mm X 40mm - 403 ml
E-3 40mm X 155mm X 40mm - 248 ml
E-4 30mm X 155mm X 40mm - 186ml
E-5 30mm X 155mm X 40mm - 186 ml
E-6 50mm X 155mm X 40mm - 318 ml

NOTE: 1/8" - 316 GRADE AUSTENISTIC STAINLESS STEEL MAY BE SUBSTITUTED FOR GLASS IN PART C.

FIG. 12. EFFLUENT CHAMBERS

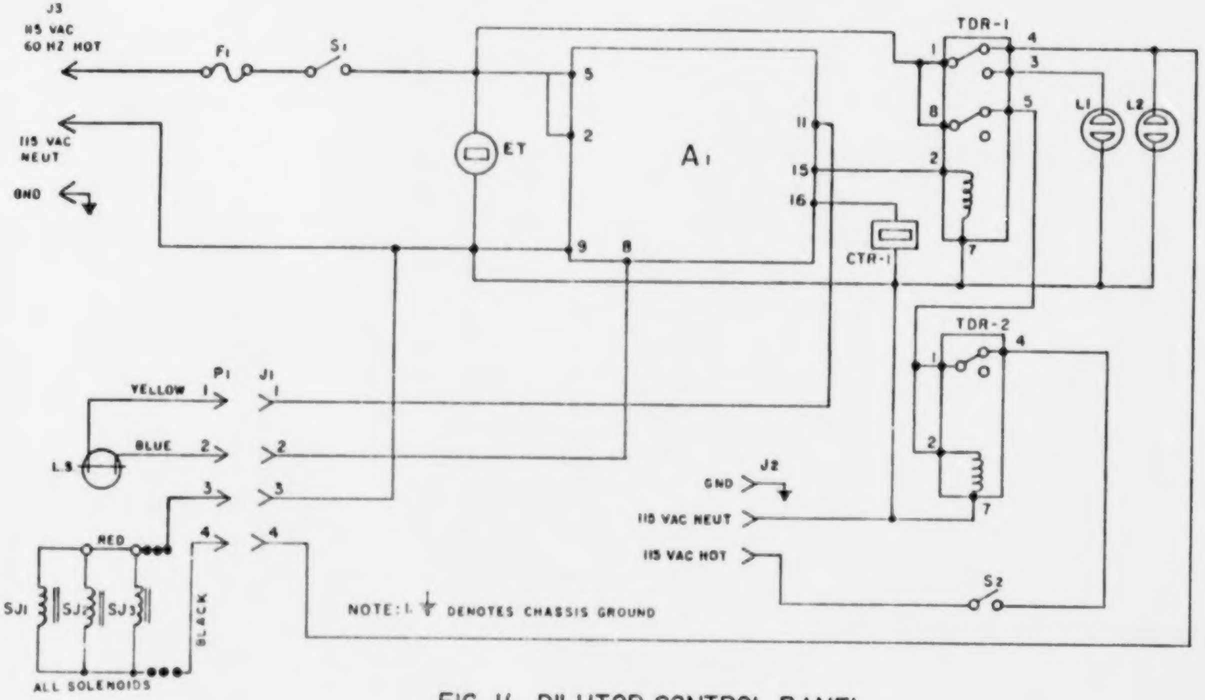

M-1
M 2
186mm
SIDE VIEW
20 mm DIAMETER HOLE
190 mm
M-3
PART M-1
END VIEW
180 mm
20 mm DIAMETER HOLE
20 mm
105 mm

INDIVIDUAL PART SIZE AND NUMBER OF PIECES USING 6mm (1/4") PLATE GLASS

M-1 180mm X 105mm-1
M-2 180mm X 105mm-1
M-3 190mm X 105mm-1 (BOTTOM PLATE)
M-4 190mm X 186mm-2 (SIDE PANELS)

(APPROXIMATE CAPACITY 3365 ml)

FIG. 13. MIXING CHAMBER


J3
115 VAC
60 HZ HOT
115 VAC
NEUT
GND
F1
S1
ET
A1
5
2
9
8
11
15
16
CTR-1
TDR-1
1
8
2
7
4
3
5
L1
L2
TDR-2
1
2
7
4
P1
J1
YELLOW
BLUE
RED
BLACK
1
2
3
4
L.S
SJ1
SJ2
SJ3
ALL SOLENOIDS
J2
GND
115 VAC NEUT
115 VAC HOT
S2

NOTE: 1. ⏚ DENOTES CHASSIS GROUND

FIG. 14. DILUTOR CONTROL PANEL.

Dilutor Control Panel Equipment List*

Designation	CKT Description	Manufacturer
A_1	Encapsulated amplifier	Cutler Hammer 1353H98C
CTR-1	Cycle counter	Rodington #P2-1006
ET	Elapsed time indicator	Courac #636W-AA H&T
F_1	Input power fuse	Little fuse 342038
J_1	Recepticle	Amphenol 91PC4F
J_2	Aux A.C. output jack	Stand. 3-prong AC Recpt.
J_3	Main input power cord	Stand. 3-prong AC maleplug
L_1	Fill indicator light	Dialco 95-0408-09-241
L_2	Emptying indicator light	Dialco 95-0408-09-241
L.S.	Level sensor (Dual Sensing Probe)	Cutler Hammer 13653H2
P_1	Plug	Amphenol 91MC4M
S_1	On-off main power switch (spst)	Cutler Hammer 7580 K7
S_2	On-off aux power switch (spst)	Cutler Hammer 7580 K7
SJ_1	Solenoid	(See Solenoid and Vacuum System equipment lists)
SJ_2	"	" " "
SJ_3	"	" " "
SJ_4 - SJ_{16}	Additional Solenoids for Solenoid Valve System	" " "
TDR-1	Time delay relay	Dayton 5x829
TDR-2	Aux time delay relay	Dayton 5x829

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*Consult local electric supply house.

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/4-78-012

2.

3. RECIPIENT'S ACCESSION NO.

4. TITLE AND SUBTITLE
METHODS FOR MEASURING THE ACUTE TOXICITY OF
EFFLUENTS TO AQUATIC ORGANISMS

5. REPORT DATE Issued January, 1978
Revised July, 1978

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7. AUTHOR(S)
William Peltier

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Athens, Georgia 30605

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Office of Research and Development
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15. SUPPLEMENTARY NOTES
Supplement to "Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters & Effluent"

16. ABSTRACT

This report describes methods for the measurement of the acute toxicity of effluents to macroinvertebrates and fish. The methods include a preliminary short-term (8-24 hr), range-finding (screening) test and a long-term (96 hr) flow-through, or alternate static, definitive test for use in determining the LC50 or EC50 of the waste. The report includes guidelines for effluent sampling and holding, facilities and equipment, dilution water, test species selection and handling, and data interpretation.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Effluents Bioassay Toxicity Industrial Wastes Sewage Water Pollution Fishes	Invertebrates Fresh Water Biology Marine Biology		6C

18. DISTRIBUTION STATEMENT
RELEASE TO PUBLIC

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Appendix B

TOXICITY TEST REPORT

I. Summary and Recommendations

A. State lethality of effluent in terms of LC50 (or EC50).

B. Review toxicity in receiving waters - IWC.

C. Recommend appropriate action, e.g. change in permit requirements, self-monitoring, use of diffusers, process alterations, alternate disposal methods, pretreatment, etc.

II. Introduction

A. Dates of study.

B. Dischanrge Serial Number

C. Principal investigators

D. Receiving water - include 7Q10 or, if tidal, with approximate salinity, amplitude range.

E. Trailer location.

F. Industrial representatives.

G. State, Federal or other observers to the study.

H. S.I.C. Code number

Plant Operation

A. Schedule of operation.

III. B. Major products and raw materials (enter SIC code).

C. Wastewater treatment schedule - periodic or continuous.

D. Daily wastewater flow rates for week prior to study and during study.

E. Retention time of effluent - arithmetic or actual.

F. Description of outfall and receiving water - submerged diffuser, etc.

G. Diagram of wastewater treatment facilities.

H. General maintenance of plant area (as pertains to the potential for contaminating runoff).

I. Description of industrial process.

J. Estimated effluent composition.

IV. Toxicity Test Methods

A. Citation of methods.

B. Explain LC50 (or EC50) data analyses used.

C. Organisms used in test: source, age and/or length and weight, health conditions.

D. Brief description of physical test facilities:

1. Test chamber capacity.
2. Aquarium turnover rate.
3. Use areation.
4. Volume of test solution (statics).

E. Chemical analyses run (wet-lab and monitor) on effluent, dilution water and samples of test solutions.

F. Type of biomonitoring test used (e.g. static, flow-through, etc.

G. Standard toxicant used.

V. Sampling Procedure

A. Effluent

1. Location and description of sampling point.

2. Note the date and time of each composite or grab sample for test or analytical purposes was recorded.

3. Note the physical and chemical parameters for which data was recorded.

4. Note types of instruments used for specific parameters and include identification labels or serial numbers.

B. Dilution water

1. Type, origin and composition if known, of dilution water used.

2. Note if a fresh/salt-water mixture was needed to achieve test salinity.

3. Note for each sample collected:

a. Location and description of sampling point.

b. Date and time.

c. DO, temperature, alkalinity, hardness (freshwater),and salinity (salt water). (In marine work, these parameters should be reported for the freshwater, saltwater, and resulting mixture if the dilution water is salinity-adjusted by mixing.)

C. Document control numbers

1. Sample tag numbers

2. Chain of custody record number

Results

VI. A. Report toxicity results of effluent - include LC50's (UCL and LCL) and list 24, 48, 72, 96 hour LC50's where appropriate (or EC50).

B. Report characteristics of effluent, dilution and receiving waters (color, odor, solids, etc.)

C. Report data, in tabular form from physical and chemical analyses recorded throughout test.

D. Report data or trends observed from continuous monitor tracings.

VII. Discussion

A. Describe events which may have affected results of toxicity tests such as aquarium turnover rate, treatment upsets, power failure, aeration, rainfall, etc.

B. Describe condition of live test organisms during and at the end of the test.

C. Relate chemical data to toxicity where applicable.

D. Calculate in-stream wastewater concentrations using dilution ratios, application factors 0.05 and 0.01 LC50 values to determine if acute or chronic toxicity exist in the receiving waters.

Appendix C

INSTRUCTIONS FOR COMPLETING THE ACUTE TOXICITY LABORATORY EVALUATION FORM

1. Laboratory or Industry - Enter the complete name of the laboratory or industry conducting the acute toxicity test.

1.a. Industry SIC Code - Enter this number and briefly describe the type of industry, raw materials used,and estimated effluent composition if available.

2. Location - Enter the address of the laboratory or industry conducting the acute toxicity test.

2.a. NPDES Permit No. - Enter the corresponding number and other necessary permit identification such as date of issuance and expiration.

3. Date - Enter date of evaluation.

4. Investigator - Enter name and title of person conducting evaluation.

5. Company Representative - Enter name of person(s) interviewed and telephone number (if available).

6. Test Method - Enter brief narrative of the test being conducted and the reference where written instructions on the methodology appears (i.e. 96-hour static bioassay; or reference: EPA 660/3-75-009 April, 1975).

7.a. Dilution Water - Source - Enter the source of the dilution water; the date and time of its collection.

7.b. Dilution Water: Chemical Analyses Performed - Enter specific chemical tests performed on dilution water if any. Also enter chemical characteristics recorded by the analyst (average and/or range values).

7.c. Dilution Water: Pretreatment - Enter a description of any pretreatment of dilution water.

8.a. Effluent Water: Source - Enter the source of the effluent to be tested, the date and time of its collection.

8.b. Effluent Water: Variability - Enter a description of the physical or chemical variability of the effluent (i.e. constant flow of effluent from a lagoon with 14-days detention time or batch process releasing effluent having variable flow and chemistry directly into the receiving water).

8.c. Effluent Water: Sampling Technique - Enter a brief description of the method used to collect the sample(s) of effluent.

8.d. Effluent Water: Holding time and Conditions - Enter the amount of time and conditions under which the test effluent is held before being used in the toxicity study.

8.e. Effluent Water: Pretreatment - Enter a description of any pretreatment of the effluent.

8.f. Effluent Water: Chemical Analyses Performed - Enter specific chemical tests performed on effluent. Also enter chemical characteristics recorded by the analyst (average and/or range values).

9.a. Test Organism: Species - Enter the common and scientific name of the test organism.

9.b. Test Organism: Life State - Enter the age, life stage, as well as length and weight (if apporpriate) of the test organism.

9.c. Test Organism: Source - Enter the specific source of the test organism; the date and time of the collection (i.e. Brown Fish Hatchery, Central City, Iowa; collected 0800 hours on January 10, 1978).

9.d. Test Organism: Holding Facilities - Enter a brief description of the facility used to hold test organisms prior to the biomonitoring study (i.e. 500-gallon Minnow-Kool tank with flow-through dechlorinated tap water).

9.e. Test Organism: Acclimation Procedure - Enter a brief description of the procedure used to acclimate the test organism to laboratory conditions prior to biomonitoring tests.

9.f. Test Organism: Treatment - Enter any observed diseases and specific treatment rendered if any. State the number of treatments and dates.

10.a. Experimental Design: Equipment Cleaning Procedure - Enter a brief description of step-by-step pre-cleaning procedure for equipment (tanks, etc.) used in biomonitoring tests. List trade name and scientific name of cleaning compounds (if available).

10.b.(1) Experimental Design: Test Chambers: Construction Material - Enter the type of material used in constructing the test chambers.

10.b.(2) Experimental Design: Test Chambers: Dimensions - Enter the specific size of the test chambers (length, width, height).

10.b.(3) Experimental Design: Test Chambers: Volume - Enter the designated volume of the test chambers as well as the specific depth and volume of solution used during the biomonitoring test.

10.b.(4) Experimental Design: Test Chambers: Volumetric Exchange Rate - Enter the rate of exchange of test solution in flow through/continuous-flow test chambers.

10.c. Experimental Design: Test concentrations - Enter a list of solution concentrations in which test organisms were exposed.

10.d. Experimental Design: Number of organisms per concentration - Enter the number of test organisms exposed to each concentration of test solution.

10.e. Experimental Design: Loading Rate - Enter the weight of test organisms per liter of test solution (i.e. 5 grams/liter).

19.f. Experimental Design: Test Temperature - Average and Range - Enter the temperature (average and range) of the solution in which test organisms are exposed during the biomonitoring study.

10.g Experimental Design: Chemical Parameters Monitored and Frequency - Enter the type of chemical tests performed and the frequency with which each chemical test is performed during the biomonitoring study.

10.h. Experimental Design: Duration and Frequency of Test - Enter the time period of the biomonitoring test and the number of times the biomonitoring test is performed each year. (Record both as "performed" and "as required in NPDES permit").

10.i. Experimental Design: Definition of adverse effect - Define the endpoint of the biomonitoring test (i.e. death).

10.j. Experimental Design: Frequency of Observations - Enter the time intervals when test organisms were observed during the biomonitoring study (i.e. observed each 12-hour period).

10.k. Experimental Design: Method of calculating EC50 or LC50 - Enter the name of the calculation procedure used and the reference citation.

10.1. Experimental Design: Special Conditions - Briefly describe test conditions not addressed elsewhere in this questionnaire (i.e. dead organisms not removed during the test; or test chambers aerated continuously with pure O_2 during test). Attach a supplement sheet if needed.

11. Methods Used for All Chemical Analyses - Enter the reference cited for the chemical analyses performed during the biomonitoring study.

12. Other Relevant Information - Enter explanations of information provided elsewhere in the Acute Toxicity Laboratory Evaluation questionnaire or other pertinent information not presented in the audit questionnaire (i.e., quality assurance program, training and experience of analyst, adequacy of laboratory equipment and facilities, etc.).

Appendix C

ACUTE TOXICITY LABORATORY EVALUATION FORM

---

1. Laboratory or industry____________________

   a. Industry or SIC Code____________________

2. Location____________________

   a. NPDES Permit No. ____________________

3. Date____________________

4. Investigator____________________

5. Company Representative____________________

6. Test Method____________________

7. Dilution Water

   a. Source____________________

   Date:__________Time____________________

   b. Chemical Analyses Performed____________________

c. Pretreatment

8. Effluent Water

a. Source

b. Variability

c. Sampling Technique

d. Holding time and conditons

e. Pretreatment

f. Chemical analyses performed

9. Test Organism

a. Species

b. Life stage

c. Source

d. Holding facilities

e. Acclimation Procedure____

____

f. Treatment (schematic or flow chart, if available)____________

____

10. Experimental Design

a. Equipment Cleaning Procedure_________________________________

____

____

b. Test Chambers

(1) Construction material___________________________________

(2) Dimensions____

(3) Volume____

(4) Volumetric exchange rate__________________________________

c. Test concentrations____

____

d. Number of organisms per concentration________________________

____

e. Loading rate____

____

f. Test temperature - average and range_________________________

____

g. Chemical parameters monitored and frequency__________________

____

____

h. Duration and frequency of test______________________________

____

i. Definition of adverse effect

j. Frequency of observations

k. Method of calculating EC50 or LC50

l. Special conditions

11. Methods used for all chemical analyses

12. Record Keeping

13. Other relevant information

Appendix D

I. General Compliance Sampling Inspection Daily Activities

The following is a suggested day-to-day inspection activities check list:

A. Day 1

1. Get power connected to mobile laboratory.
2. Level and stabilize laboratory.
3. Collect dilution water.
4. Begin acclimation (approximately 2 tank volumes in 24 hours) (Appendix A, page 13).
5. Set up static range finding test (Appendix A, page 19).
6. Make necessary entries in logbooks and fill in necessary forms.

B. Day 2

1. Check results of range finding test and make necessary logbook entries.
2. Assemble dilution board and delivery system.
3. Calibrate dilution board.
4. Activate diluter and begin filling test tanks.
5. Cease flow to acclimation tank.
6. If a composite sample is to be used for the static test; the compositer should be set up this day.
7. Collect dilution water.
8. Make all necessary logbook entries.

C. Day 3

1. Check all systems to ascertain that all have worked overnight.

2. Check temperatures to see if the acclimation temperature and test temperature are approximately the same.

3. Start the pump in the circulating water bath and turn the thermal equilizing unit on.

4. Collect the sample for the static test whether it be grab or composite.

5. Set up static test tanks.

6. Perform temperature, D.O., pH, and conductivity readings in all test containers.

7. Introduce the test organisms to both the static and flow-through test containers.

8. Collect additional dilution water.

D. Days 4, 5, and 6

1. Check all systems to ascertain that all have worked overnight.

2. Record test organism mortality in all test containers and remove dead organisms where appropriate.

3. Perform length and weight measurements on dead fish (make necessary logbook entries).

4. Calibrate the appropriate meters and take meter readings.

5. Collect dilution water.

6. When scheduled, conduct a compliance biomonitoring evaluation inspection (see section IV C.)

7. Make all necessary logbook entries.

E. Day 7

1. Check all systems to ascertain that all have worked overnight.

2. Record test organism mortality in all test containers and remove the dead organisms where appropriate.

3. Calibrate the appropriate meters and take meter readings.

4. Recalibrate diluter board.

5. Make all necessary entries in logbooks.

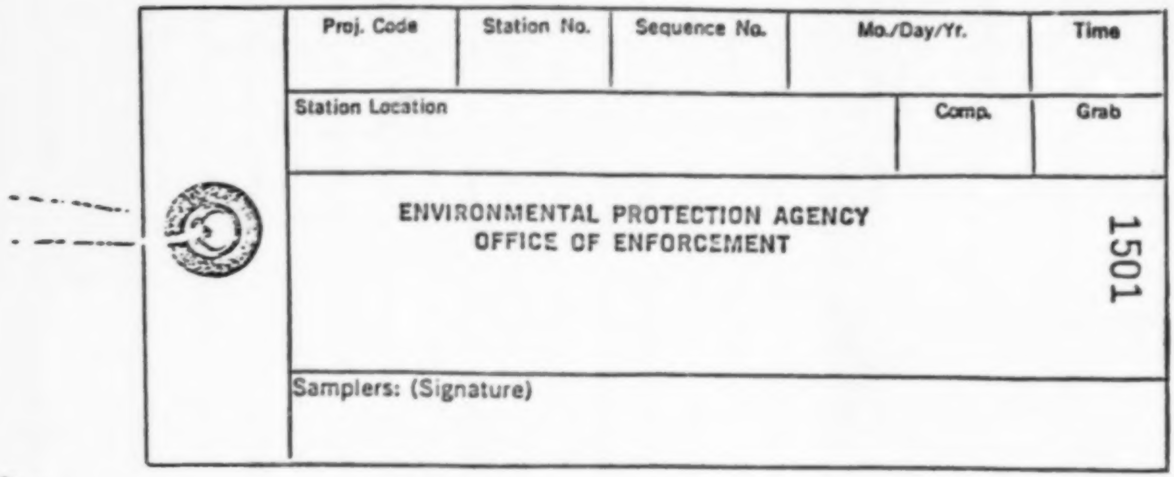
6. Dismantle laboratory and secure equipment.

7. Inform permittee of your departure and sign out with gate security guard.

Appendix E

Sample Tags and Chain of Custody Form

SAMPLE TAG*



Proj. Code	Station No.	Sequence No.	Mo./Day/Yr.	Time

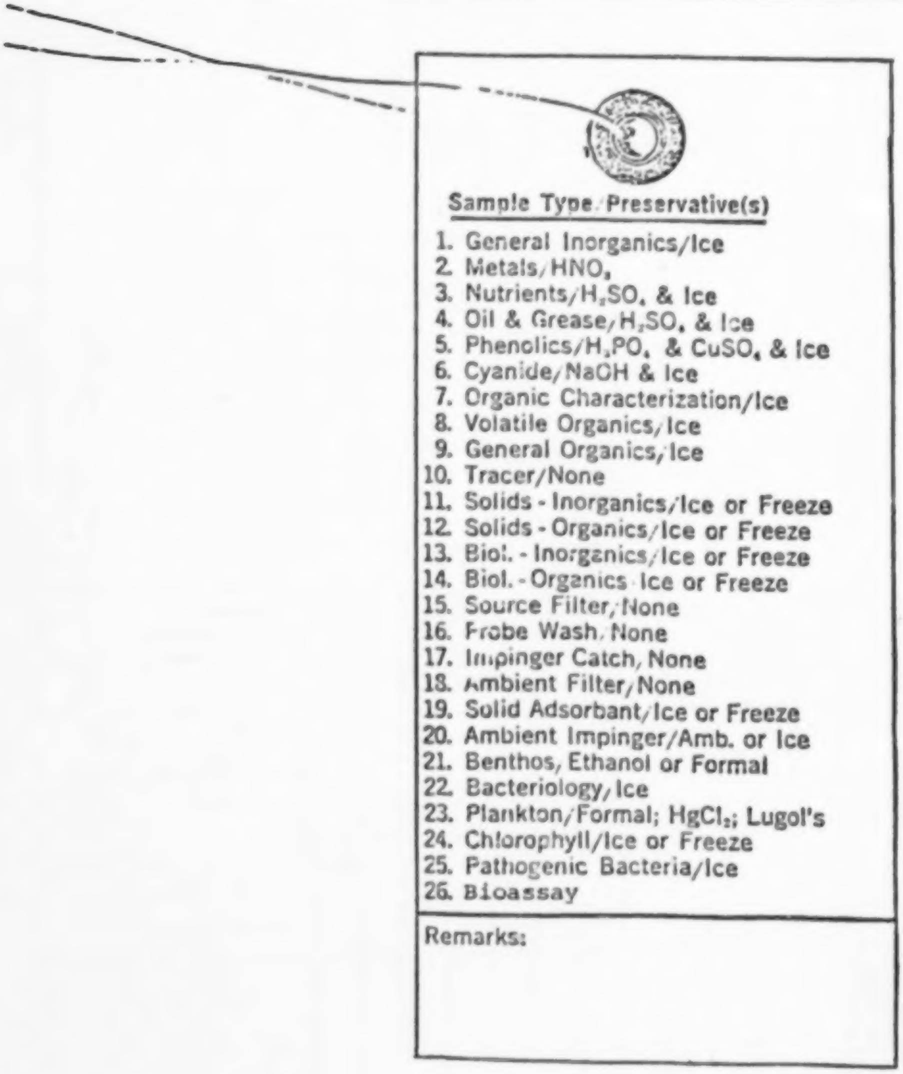
Station Location	Comp.	Grab

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF ENFORCEMENT

1501

Samplers: (Signature)

obverse



Sample Type/Preservative(s)

1. General Inorganics/Ice
2. Metals/HNO_3
3. Nutrients/H_2SO_4 & Ice
4. Oil & Grease/H_2SO_4 & Ice
5. Phenolics/H_3PO_4 & $CuSO_4$ & Ice
6. Cyanide/NaOH & Ice
7. Organic Characterization/Ice
8. Volatile Organics/Ice
9. General Organics/Ice
10. Tracer/None
11. Solids - Inorganics/Ice or Freeze
12. Solids - Organics/Ice or Freeze
13. Biol. - Inorganics/Ice or Freeze
14. Biol. - Organics/Ice or Freeze
15. Source Filter/None
16. Probe Wash/None
17. Impinger Catch/None
18. Ambient Filter/None
19. Solid Adsorbant/Ice or Freeze
20. Ambient Impinger/Amb. or Ice
21. Benthos/Ethanol or Formal
22. Bacteriology/Ice
23. Plankton/Formal; $HgCl_2$; Lugol's
24. Chlorophyll/Ice or Freeze
25. Pathogenic Bacteria/Ice
26. Bioassay

Remarks:

reverse

*Modified and adopted from: NEIC Policies and Procedures Manual, May 1978. - Denver, CO

ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF ENFORCEMENT

CHAIN OF CUSTODY RECORD*

Appendix E
Page 2 of 2

Proj. No. | Project Name

SAMPLERS: *(Signature)*

					SAMPLE TYPE																												NUMBER OF CONTAINERS	Remarks
STA. NO.	SEQ. NO.	DATE	TIME	STATION LOCATION	1.General Inorganics	2. Metals	3. Nutrients	4. Oil & Grease	5. Phenolics	6. Cyanide	7. Organic Characterization	8. Volatile Organics	9. General Organics	10. Tracer	11. Solids - Inorganics	12. Solids - Organics	13. Biol - Inorganics	14. Biol - Organics	15. Source Filter	16. Probe Wash	17. Impinger Catch	18. Ambient Filter	19. Solid Adsorbant	20. Ambient Impinger	21. Benthos	22. Bacteriology	23. Plankton	24. Chlorophyll	25. Pathogenic Bacteria	26. Bioassay	27.	28.		
																					TOTAL NO. OF CONTAINERS													

Relinquished by: *(Signature)*	Date/Time	Received by: *(Signature)*	Relinquished by: *(Signature)*	Date/Time	Received by: *(Signature)*
Relinquished by: *(Signature)*	Date/Time	Received by: *(Signature)*	Relinquished by: *(Signature)*	Date/Time	Received by Courier *(Signature)*
Relinquished by Courier *(Signature)*	Date/Time	Received by Mobile Lab *(Signature)*	Relinquished by Mobile Lab *(Signature)*	Date/Time	Received by Courier *(Signature)*
Method of Shipment:		Shipped by: *(Signature)*	Courier from Airport *(Signature)*	Received for Laboratory by: *(Signature)*	Date/Time

Distribution: Original Accompanies Shipment: Copy to Coordinator Field Files

*Modified and adopted from; NEIC Policies And Procedures Manual, May 1978. - Denver, CO

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APPENDIX F

DOCUMENT CONTROL

The goal of the NEIC Document Control Program is to assure that all documents for a specific project issued to or generated by NEIC personnel will be accountable when the project is completed. This program includes a serialized document number system, a document inventory procedure, and a central filing system, all under the supervision of a Document Control Officer (DCO).

Accountable documents used or generated by NEIC employees include items such as logbooks, field data records, correspondence, sample tags, graphs, chain of custody records, bench cards and photos (see page II-27 for a more complete list). Each document bears a serialized number and is listed, with the number, in a project document inventory assembled by each Branch at the project's completion.

Unless prohibited by weather, waterproof ink is used in recording all data on serialized accountable documents.

SERIALIZED DOCUMENTS

The DCO is responsible for assigning the necessary serialized NEIC documents to project personnel for field activities. Once a Project Coordinator is appointed, all field logbooks, field data records, field laboratory logbooks, sample tags and chain-of-custody records are assigned to this person. The Coordinator is responsible for ensuring that a sufficient supply of documents is obtained for an investigation and that these documents are properly distributed to the appropriate personnel. The DCO provides the Project Coordinator with a list of all serialized project documents that were assigned to personnel for field activities.

PROJECT LOGBOOKS

The logbook of the Project Coordinator will document the transfer of logbooks to the individuals who have been designated to perform specific tasks on the survey. All pertinent information should be recorded in these logbooks from the time each individual is assigned to the project until the project is completed.

Logbook entries should be dated, legible and contain accurate and inclusive documentation of an individual's project activities. Since the logbook forms the basis for the later written reports, it must contain only facts and observations. Language should be objective, factual and free of personal feelings or other terminology which might prove inappropriate. Entries made by individuals other than the person to whom the logbook was assigned are dated and signed by the individual making the entry.

Field analysts who conduct their assigned project analyses in a mobile laboratory are assigned a Branch logbook by the Chemistry Branch. In addition to information documenting the analysis performed, field analysts document in their logbooks the date and results of any calibration of mobile laboratory equipment. A record is also kept of any incidents related to the survey; for example, the electricity going off in the lab, tampering with government vehicles or equipment, etc. When appropriate, visitors to the mobile lab, such as facility personnel, are noted in the logbook.

All project logbooks are the property of NEIC and are to be turned over to the Project Coordinator when a survey assignment has been concluded.

FIELD DATA RECORDS

Where appropriate, serialized Field Data Records (in the form of individual sheets or bound logbooks) are maintained for each survey sampling station or location. The Project Coordinator numbers the FDR's with the appropriate project code and station number. All in-situ measurements and field observations are recorded in the FDR's with all pertinent information necessary to explain and reconstruct sampling operations. Each page of a Field Data Record is dated and signed by all individuals making entries on that page. The Coordinator and the field team on duty are responsible for ensuring that FDR's are present during all monitoring activities and are stored safely to avoid possible tampering.

SAMPLE INDENTIFICATION DOCUMENTS

Assignment of all serialized sample tags to field personnel is recorded in the Project Coordinator's logbook. Individuals are accountable for each tag assigned to them until it has been filled out, attached to a sample, and transferred to another individual with the corresponding Chain-of-Custody Record. At no time are any sample tags to be discarded; if any of these forms are lost, voided or damaged, it is noted in the appropriate FDR or logbook immediately upon discovery. Tags attached to those samples split with the facility or another government agency will be accounted for as described below.

At the completion of any reconnaissance or field-sampling investigation, all unused sample tags are returned to the Project Coordinator by the individual to whom they were originally assigned. This individual lists the serial numbers of the returned items in the Coordinator's logbook and signs and dates the transfer.

CHAIN-OF-CUSTODY RECORDS

All serialized Chain-of-Custody Records are assigned and accounted for in a manner similar to that for the sample tags as described above. When samples are transferred from a field sampler or courier to field laboratory personnel, the analyst, after signing, retains the white (original) custody record and files it in a safe place. The copy of the custody record is returned to the Project Coordinator. A similar procedure is followed when dispatching samples via common carrier, mail, etc., except that the original accompanies the shipment and is signed and retained by the receiving laboratory sample custodian.

When samples are split with the facility or another government agency, the separate custody record that is prepared (see page 24) is labeled to indicate this. In addition, the serial numbers from all the tags are recorded on the custody record. The person relinquishing the samples to the facility or agency should request the signature of a representative of the appropriate party, acknowledging receipt of the samples. If a representative is unavailable or refuses to sign, this is noted in the "received by" space. When appropriate, as in the case where the representative is unavailable, the custody record should contain a statement that the samples were delivered to the designated location at the designated time. The copy of the custody record may be given to the facility or agency upon request; all white originals are returned to the Project Coordinator.

ANALYST, INSTRUMENT AND SAMPLE ENTRY LOGBOOKS

Logbooks and data sheets that are used for various purposes (chemical or biological analyses, equipment calibration, etc.) within the NEIC laboratories are not handled by the DCO, but rather are accountable by practices instituted by individual Branches.

All laboratory observations and calculations not recorded on serialized bench cards, instrument graph printouts, etc., are entered in serialized logbooks assigned by a Branch file custodian. The logbook should contain information sufficient to recall and describe succinctly each step of the analysis performed should the analyst be required to testify in subsequent enforcement proceedings. Sufficient detail should be provided to enable others to reconstruct the analysis should the analyst not be available to do so. Any irregularities observed during the testing process should be noted. If, in the technical judgment of the analyst, it is necessary to deviate from a particular analytical method, the deviation shall be properly justified and documented.

When an individual is assigned a logbook for use on a variety of projects, each page contains information about only one project and is labeled with the project code, dated, and signed by the individual. All bench cards, instrument printouts, and other separate documents are labeled similarly. Notes (taken at meetings, from research articles, etc.) which do not relate to a particular NEIC project shall not be kept in the assigned logbook. When a laboratory logbook is completed, it is returned to the Branch file custodian and a new logbook is issued. The custodian or other appropriate staff member maintains an inventory sheet for the logbook, listing the project code for each page. These books that have been completed and turned in are used for reference purposes only.

Where applicable, the Branch file custodian issues a serialized instrument logbook in which all information relating to calibration and maintenance of a particular laboratory instrument is recorded. A serialized sample entry logbook is used in the laboratory to record the entry of the samples to the laboratory or laboratory instrument for analysis. Again, each page should contain information about one project only.

PHOTOGRAPHS

When movies, slides or photographs are taken which visually show the effluent or emission source and/or any monitoring locations, they are numbered to correspond to logbook entries. The name of the photographer, date, time, site location, and site description are entered sequentially in the logbook as photos are taken. Once developed, the slides or photographs should be serially numbered corresponding to the logbook descriptions.

CORRECTIONS TO DOCUMENTATION

As previously noted, unless prohibited by weather conditions, all documentation in logbooks, FDR's, sample tags, custody records and other data sheets are filled out with waterproof ink. None of the accountable serialized documents listed above are to be destroyed or thrown away even if they are illegible or contain inaccuracies which required a replacement document.

If an error is made in a project logbook assigned to one individual, that individual may make corrections simply by crossing a line through the error and entering the correct information. Changes made subsequently are dated and initialed. If an error is discovered on a sample tag, custody record or FDR, when possible the person who made the error should correct it. Corrections or insertions are made by inserting the word or abbreviation for "corrected," the date, and the correcting person's initials beside the correction. The procedure applies to words or figures inserted or added to a prior recorded statement.

If a sample tag is lost in shipment, or a tag was never prepared for a sample(s), or a properly tagged sample was not transferred with a formal NEIC Chain-of-Custody Record, the following procedure applies. A written statement is prepared detailing how the sample was collected, air-dispatched or hand-transferred to the field or NEIC laboratory. The statement should include all pertinent information, such as entries in field logbooks regarding the sample, whether the sample was in the sample collector's physical possession or in a locked compartment until hand-transferred to the laboratory, etc. Copies of the statement are distributed to the Project Coordinator, the Assistant Director for Technical Programs and the appropriate Branch project files.

CONSISTENCY OF DOCUMENTATION

Before releasing any analytical sample results to the Project Coordinator, the Chemistry and/or Biology Branches assemble and cross-check information on corresponding sample tags, custody records, bench cards, analyst logbooks and sample entry logbooks to ensure that data pertaining to each particular sample is consistent through out the record. A statement that all project evidentiary data in the Branch's possession has been accounted for accompanies the transfer of any analytical data from the NEIC laboratories to the Project Coordinator.

The Project Coordinator then conducts a cross-check of evidentiary data in his possession (FDR's, logbooks, custody records, etc.) to ensure that information recorded corresponds to information from each of the Branch laboratories and is consistent throughout the project record.

DOCUMENT NUMBERING SYSTEM AND INVENTORY PROCEDURE

In order to provide document accountability to the appropriate individuals, each of the document categories discussed above features a unique serialized number for each item within the category. Logbooks, FDR's, sample tags and custody records are serially numbered by the DCO before assignment to project personnel. The logbooks and FDR's are usually given a five-digit number, with the project code as the first three digits followed by a two-digit document number. Sample tags and custody records are labeled with a four digit document number and the project code appears elsewhere on the document. All Branch documentation not covered by the above (logbooks, data sheets, graphs, etc.) are uniquely and serially numbered using the project code as part of the number when appropriate.

All other documents (such as recorder graph paper, data calculation sheets, memos, correspondence, photos, etc.) which are generated during a project are sequentially numbered with the project code, the Branch initials and a serialized number (e.g., 707-CH-01), usually at the time the Branch file is assembled.

BRANCH FILES

After a Branch has completed its work for a particular investigation, all documents generated from that project should be assembled in the Branch file. Individuals may retain clean (no handwritten comments) copies of documents for their personal files but only after personally verifying that the original or similar copy is in the Branch file. The Chief of each Branch in Technical Programs is responsible for assuring the collection, assembly, and inventory of all documents relative to a particular project at the time the project objectives are completed. The file then becomes accountable. Any records leaving the file must be signed out.

CENTRAL FILE

When the NEIC has completed the project objectives, all inventoried Branch file documents are reviewed and submitted to the Central File by each Branch Chief. By this time each document will have been labeled with a unique serialized number as specified above. The format of the Central File covers the following document classes:

A. Project Logbooks
B. Field Data Records
C. Sample Identification Documents
D. Chain-of-Custody Records
E. Analytical Logbooks, Lab Data, Calculations, Bench Cards, Graphs, etc.
F. Correspondence

  1. Intra-office
  2. EPA
  3. Industry
  4. Record of Confidential Material

G. Report Notes, Calculations, etc.; Drafts
H. References, Literature
I. Sample (on-hand) Inventory
J. Check-out Logs
K. Litigation Documents
L. Miscellaneous - photos, maps, drawings, etc.

Once deposited in the Central File, documents may only be checked out through the DCO or designated representative.

REPORTS

All draft reports are numbered and accountable. They are stamped DRAFT REPORT FOR AGENCY REVIEW ONLY, DO NOT DUPLICATE on the cover page. The author is responsible for disseminating draft reports for internal NEIC review, and preparing a memorandum for the Assistant Director for Technical Programs to transmit copies to Regional Offices, Headquarters, etc. All draft copies of the report are to be returned to the author. Once comments have been incorporated and the final report has been prepared, all draft copies are disposed of. However, Regional Offices may retain a copy of the draft report with their comments until they receive the final report at which time the draft will be returned to the NEIC.

LITIGATION DOCUMENTS

Any court documents, litigation reports, letters, memos, etc. from the Chief, Enforcement Specialist Office, EPA Regional Office(s), State Pollution Control Offices, etc., which discuss legal matters or strategies, should be placed in a separate file folder (see Central File format) which is reviewed by the Enforcement Specialist Office at the appropriate time.

CONFIDENTIAL INFORMATION

Any information received by NEIC with a request of confidentiality is handled as "confidential." A separate, locked file is maintained in the Central File room for the segregation and storage of all confidential and trade-secret information. Upon receipt by NEIC, this information is directed to and recorded in the Confidential Inventory Log by the DCO. The information is then made available to NEIC personnel, but only after it has been logged out. The information should be returned to the locked file at the conclusion of each working day. Confidential information may not be reproduced except upon approval by and under the supervision of the DCO. Any reproduction should be kept to an absolute minimum. The DCO will enter all copies into the document control system and apply the same requirements as for the original. In addition, this information may not be entered

Appendix G

DEFINITIONS

Acclimation - The process of adjusting to environmental changes.

Acute Toxicity - Short-term effect of a toxicant on test organisms. Death is the end point in acute toxicity tests.

Announced Inspection - Is the type of inspection in which the permittee is made aware of the exact dates in which the inspection is to take place.

Application Factor (AF) - Is an indirect method used in estimating the maximum allowable toxicant concentration (MATC) or the safe concentration (SC). Application factors are generally estimated but their true value is the ratio between MATC and the incipient LC50 or: $AF = \frac{\text{MATC}}{\text{Incipient LC 50}}$ (See pages 686 and 689 of the 14th Edition of Standard Methods).

Audit (or Performance Audit Inspection-PAI) - Is a non-sampling type inspection that assesses all the elements of a permittee's self-monitoring program while they are being performed. This includes review of quality assurance, sample collection, files and analytical laboratory data.

Bioassay - Is a test which utilizes any biological system to detect or measure the presence or effect of one or more substances, waste, or environmental factors alone or in combination.

Biomonitoring - For this manual's purposes, biomonitoring refers to acute toxicity bioassays performed for the NPDES program.

Chain of Custody - Includes all the administrative procedures directed at protecting and certifying the integrity and therefore the acceptability of evidence in a legal proceeding.

Chronic Toxicity - Long-term effect of a toxicant on test organisms. Effective concentration (EC) as determined by sublethal behavioral or physiological response of the test species is the end point.

Composite Sample - The type of sample made out of several discrete samples collected either at equal time intervals or proportional to the flow rate.

over the compositing period (see pages 28 to 32 of EPA's NPDES Compliance Sampling Manual, June 1977).

Definitive Test - Full scale bioassay consisting of at least five different concentrations of effluent in and exponential series with each concentration and control being tested against no less than 20 organisms of a given species.

Dilution Water - Mixing water to be used for preparing the different test exponential dilution series of the effluent. This water is usually collected from a point as close as possible but away from the zone of influence of the effluent's discharge.

Discharge Monitoring Report (DMR) - Form used by NPDES permittees to report the results of their self-monitoring which is required by their NPDES permit, must be submitted to the respective NPDES permitting authority at least quarterly. At present EPA Form 3320-1 (Rev. 10-77) is used for reporting purposes.

Document Control - Administrative procedures used for the purpose of tracking and maintaining adequate records of all documents issued by or generated by a particular program (see Appendix G of this manual).

EC50 - Or median effective concentration is the concentration producing a specific response, other than death, in 50% of the test organisms. Responses can be behavioral, a developmental abnormality, or a deformity.

Effluent - For the purposes of this manual, is an outflow from a point source with some of its physical, chemical, and biological parameters being regulated by an NPDES permit.

Evaluation Inspections - Inspections that involve a review and evaluation of all self-monitoring and better records required by an NPDES permit.

Flow-through Bioassay - Or continuous flow bioassay is the type of test where different concentrations of the effluent are prepared by mixing it with adequate quality dilution water and then tested by allowing such effluent concentrations to flow at predetermined rates into chambers containing the test organism.

Grab Sample - Individual sample collected over a period of time not to exceed 15 minutes.

Incipient Lethal Level - The concentration at which acute toxicity ceases, that is, the concentration at which 50% of the test organism's population can live for an indefinite time.

In-stream Waste Concentration (IWC) - The concentration (expressed as percent) of permittee's waste in the receiving stream at the 7Q10.

Lethal Units (LU) - Normally given in LU per gallon (LU/g) are defined as $LU/g = \frac{100\%}{LC50\%}$

Maximum Allowable Toxicant Concentration (MATC) - The concentration of toxic waste that may be present in the receiving water without causing significant harm to its productivity and uses. Usually determined by a long-term bioassay (from egg to egg or beyond).

Monitoring - In this manual, the term refers to overview by actual sampling and/or evaluation of NPDES permittee's compliance with permit conditions.

NPDES or National Pollutant Discharge Elimination System - Refers to permit system developed by EPA under the authority granted by section 402 of the Clean Water Act.

Quality Assurance (QA) - For this manual's purposes refers to all scientific tests and administrative procedures used to ensure the scientific and legal validity of results obtained from biomonitoring tests.

Range Finding Test - Refers to a short-term (8-24 hours) flow-through or static bioassay (usually static) used for determining the approximate concentrations, above and below the LC50, to be used in the definite test. In this test, groups of five organisms are exposed from three to five widely spaced effluent dilutions.

Sampling Point - Particular site whose location may be specified in a permit and from which effluent samples are to be collected for testing and evaluation.

Standard Toxicant - Toxic reference material used for QA purposes in the biomonitoring program. Its main functions are to determine the reproducibility of test results and differences in sensitivity among batches of test organisms.

Unannounced Visits - Inspections where the permittee is not given notice of the exact date on which the inspection will take place except through a general 308 letter.

7Q10 - The once in 10 year, seven day consecutive low flow of a stream.

Appendix H

FACTORS USEFUL IN SELECTING CANDIDATES FOR INSPECTION

Any consideration of factors useful in prioritizing biomonitoring resources for enforcement purposes must involve two types of activities:

° Problem area monitoring - a reaction program with candidates selected on the basis of known problems.

Several factors may trigger the need for a compliance biomonitoring inspection at an NPDES permitted facility including:

° Fish kills in receiving waters

° Permit violations associated with discharges containing potentially toxic substances.

° Citizen complaints

° Questionable or inadequate self-monitoring data

° Biological monitoring studies indicating that a receiving body of water is not supporting a productive and diverse biota when it normally should.

The actual use of resources to investigate problem areas is normally based on the judgment of the enforcing agency on a case by case basis.

- Preventive monitoring - a carefully planned long-term program aimed at detecting potential problems before they occure and/or detecting problems that are not readily apparent. Problem area biomonitoring activity has first priority.

A preventive monitoring program should result in a lower incidence of problems related to the discharge of toxic substances.

The following problems should be considered when selecting sites for bioassay inspections:

- Industrial category - this is the first order of importance in evaluating the potential for discharge of toxic substances by a permittee. This also includes municipals receiving industrial wastes.

- Instream waste concentration (IWC) - for a given industrial type the potential for toxicity of a waste discharge to aquatic life is a function of concentration. For flowing waters a measure, or estimate of the 7Q10 (see section VI A.) flow should be used in calculating the IWC. In lakes and estuaries the permittees should be ranked on the basis of the volume of the waste discharged. In most cases, any discharges to lakes or estuaries should be considered more critical than discharges to rivers.

° Quality of receiving water - As a first approximation the State-Federal water quality standards for the receiving waters should be determined and the receiving waters ranked in order of highest use.

° Status of permit - The permittees should be ranked on the basis of the time remaining before an existing permit is to expire. Those permits whose expiration date is earliest would receive highest priority.

APPENDIX I

Conduct of Inspections After the Barlow's Decision; Development of Neutral Administrative Inspection Schemes

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

11 APR 1979

OFFICE OF ENFORCEMENT

MEMORANDUM

TO: Deputy Assistant Administrators for Enforcement

FROM: Assistant Administrator for Enforcement

SUBJECT: Development of Neutral Administrative Inspection Schemes

It is essential that all enforcement programs develop, as soon as possible, neutral schemes for the conduct of all inspections. This requirement is mandated by the decision of the Supreme Court in *Marshall v. Barlow's, Inc.*, U. S. , 98 S. Ct. 1816 (1978). As you know, the *Barlow's* decision addressed the issue of the need for and use of warrants in conducting administrative inspections under various regulatory schemes. The Court stated, in general, that a warrant was necessary when requested by the owner or person-in-charge of the establishment to be inspected, but that the warrant need not be based on a showing of criminal probable cause. Rather, a warrant would be issued if it could be shown that the establishment was being inspected pursuant to a neutral administrative scheme.

The Department of Justice and the Office of General Counsel agree that there is an urgent need for each compliance monitoring program to develop a neutral administrative scheme for inspections, and that such schemes must be put into writing. If the schemes are not documented until an inspection warrant is sought, they will appear to be *post hoc* rationalizations for ill-conceived or harassing inspectional programs and will not be favored by the courts.

I believe that most of our compliance monitoring programs have developed inspection programs which utilize neutral criteria. In some instances, however, the total neutral scheme for each program has not been formally documented. Examples of neutral criteria which could satisfy the requirements of *Barlow's* are random selection; inspecting all or a fixed percentage of certain types of facilities on an annual basis; ranking inspections by the amount of controlled chemical substances manufactured, processed or distributed in commerce; re-inspection of establishments which exhibited prior violations; and other reasonable factors that show that the establishment being inspected has not been selected for any arbitrary or invidious reason. The Office of General Counsel will cooperate with the Office of Enforcement in assuring that the neutral administrative schemes developed by each program are compatible with the *Barlow's* decision.

I am sending to the Regions a guidance document, in which you have already concurred, entitled "Conduct of Inspections after the Barlow's Decision." The document informs the Regions that inspections, other than those based upon probable cause, must be based on a neutral administrative scheme, and that such schemes are being prepared at Headquarters for each compliance monitoring program. As new enforcement programs are developed, a neutral administrative inspection scheme must be developed in writing by the time of program implementation. For programs already in existence, a written neutral administrative scheme should be developed, and submitted to the Office of General Counsel, by May 31, 1979.

Marvin B. Durning

Marvin B. Durning

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

11 APR 1979

MEMORANDUM

OFFICE OF ENFORCEMENT

TO: Regional Administrators
Surveillance and Analysis Division Directors
Enforcement Division Directors

FROM: Assistant Administrator
for Enforcement

SUBJECT: Conduct of Inspections After the Barlow's Decision

I. Summary

This document is intended to provide guidance to the Regions in the conduct of inspections in light of the recent Supreme Court decision in *Marshall v. Barlow's, Inc.*, ___ U.S. ___, 98 S. Ct. 1816 (1978). The decision bears upon the need to obtain warrants or other process for inspections pursuant to EPA-administered Acts.

In *Barlow's*, the Supreme Court held that an OSHA inspector was not entitled to enter the non-public portions of a work site without either (1) the owner's consent, or (2) a warrant. The decision protects the owner against any penalty or other punishment for insisting upon a warrant.

In summary, *Barlow's* should only have a limited effect on EPA enforcement inspections:

- Inspections will generally continue as usual;
- Where an inspector is refused entry, EPA will seek a warrant through the U.S. Attorney;
- Sanctions will not be imposed upon owners of establishments who insist on a warrant before allowing inspections of the non-public portions of an establishment.

The scope of the *Barlow's* decision is broad. It affects all current inspection programs of EPA, including inspections conducted by State personnel and by contractors. The Agency's procedures for inspections, particularly where entry is denied, were largely in accord with the provisions of *Barlow's* before the Supreme Court issued its ruling. Nevertheless, a number of changes in Agency procedure are warranted. Thus, it is important that all personnel involved in the inspection process be familiar with the procedural guidelines contained in this document.

This document focuses on the preparation for and conduct of inspections, including (1) how to proceed when entry is denied, (2) under what circumstances a warrant is necessary, and (3) what showing is necessary to obtain a warrant.

II. Conduct of Inspections

The following material examines the procedural aspects of conducting inspections under EPA-administered Acts. Inspections are considered in three stages: (1) preparation for inspection of premises, (2) entry onto premises, and (3) procedures to be followed where entry is refused.

A. Preparation

Adequate preparation should include consideration of the following factors concerning the general nature of warrants and the role of personnel conducting inspections.

(1) Seeking a Warrant Before Inspection

The Barlow's decision recognized that, on occasion, the Agency may wish to obtain a warrant to conduct an inspection even before there has been any refusal to allow entry. Such a warrant may be necessary when surprise is particularly crucial to the inspection, or when a company's prior bad conduct and prior refusals make it likely that warrantless entry will be refused. Pre-inspection warrants may also be obtained where the distance to a U.S. Attorney or a magistrate is considerable so that excessive travel time would not be wasted if entry were denied. At present, the seeking of such a warrant prior to an initial inspection should be an exceptional circumstance, and should be cleared through Headquarters. If refusals to allow entry without a warrant increase, such warrants may be sought more frequently. (For specific instructions on how to obtain a warrant, see Part D.)

(2) Administrative Inspections v. Criminal Investigations

It is particularly important for both inspectors and attorneys to be aware of the extent to which evidence sought in a civil inspection can be used in a criminal matter, and to know when it is necessary to secure a criminal rather than a civil search warrant. There are three basic rules to remember in this regard: (1) If the purpose of the inspection is to discover and correct, through civil procedures, noncompliance with regulatory requirements, an administrative inspection (civil) warrant may be used; (2) if the inspection is in fact intended, in whole or in part, to gather evidence for a possible criminal prosecution, a criminal search warrant must be obtained under Rule 41 of the Federal Rules of Criminal Procedure; and (3) evidence obtained during a valid civil inspection is generally admissible in criminal proceedings. These principles arise from the recent Supreme Court cases of Marshall v. Barlow's, Inc., supra; Michigan v. Tyler, ____U.S.____, 98 S.Ct. 1942 (1978); and U.S. v. LaSalle National Bank, ____U.S.____, 57 L. Ed. 2d 221 (1978). It is not completely clear whether a combined investigation for civil and criminal violations may be properly conducted under a civil or "administrative" warrant, but we believe that a civil warrant can properly be used unless the intention is clearly to conduct a criminal investigation.

(3) The Use of Contractors to Conduct Inspections

Several programs utilize private contractors to aid in the conduct of inspections. Since, for the purpose of inspections, these contractors are agents of the Federal government, the restrictions of the Barlow's decision also apply to them. If contractors are to be conducting inspections without the presence of actual EPA inspectors, these contractors should be given training in how to conduct themselves when entry is refused. With respect to obtaining or executing a warrant, an EPA inspector should always participate in the process, even if he was not at the inspection where entry was refused.

(4) Inspections Conducted by State Personnel

The Barlow's holding applies to inspections conducted by State personnel and to joint Federal/State inspections. Because some EPA programs are largely implemented through the States, it is essential that the Regions assure that State-conducted inspections are conducted in compliance with the Barlow's decision, and encourage the State inspectors to consult with their legal advisors when there is a refusal to allow entry for inspection purposes. State personnel should be encouraged to contact the EPA Regional Enforcement Office when any questions concerning compliance with Barlow's arise.

With regard to specific procedures for States to follow, the important points to remember are: (1) The State should not seek forcible entry without a warrant or penalize an owner for insisting upon a warrant, and (2) the State legal system should provide a mechanism for issuance of civil administrative inspection warrants. If a State is enforcing an EPA program through a State statute, the warrant process should be conducted through the State judicial system. Where a State inspector is acting as a contractor to the Agency, any refusal to allow entry should be handled as would a refusal to an Agency inspector as described in section II.B.3. Where a State inspector is acting as a State employee with both Federal and State credentials, he should utilize State procredures unless the Federal warrant procedures are more advantageous, in which case, the warrant should be sought under the general procedures described below. The Regions should also assure that all States which enforce EPA programs report any denials of entry to the appropriate Headquarters Enforcement Attorney for the reasons discussed in section II.B.4.

B. Entry

(1) Consensual Entry

One of the assumptions underlying the Court's decision is that most inspections will be consensual and that the administrative inspection framework will thus not be severely disrupted. Consequently, inspections will normally continue as before the Barlow's decision was issued. This means that the inspector will not normally secure a warrant before undertaking an inspection but, in an attempt to gain admittance, will present his credentials and issue a notice of inspection where required. The establishment owner may complain about allowing an inspector to enter or otherwise express his displeasure with EPA or the Federal government. However, as long as he allows the inspector to enter, the entry is voluntary and consensual unless the inspector is expressly told to leave the premises. On the other hand, if the inspector has gained entry in a coercive manner (either in a verbal or physical sense), the entry would not be consensual.

Consent must be given by the owner of the premises or the person in charge of the premises at the time of the inspection. In the absence of the owner, the inspector should make a good faith effort to determine who is in charge of the establishment and present his credentials to that person. Consent is generally needed only to inspect the non-public portions of an establishment - i.e., any evidence that an inspector obtains while in an area open to the public is admissible in an enforcement proceeding.

(2) Withdrawal of Consent

The owner may withdraw his consent to the inspection at any time. The inspection is valid to the extent to which it has progressed before consent was withdrawn. Thus, observations by the inspector, including samples and photographs obtained before consent was withdrawn, would be admissible in any subsequent enforcement action. Withdrawal of consent is tantamount to a refusal to allow entry and should be treated as discussed in section II.B.3. below, unless the inspection had progressed far enough to accomplish its purposes.

(3) When Entry is Refused

Barlow's clearly establishes that the owner does have the right to ask for a warrant under normal circumstances.[1] Therefore, refusal to allow entry for inspectional purposes will not lead to civil or criminal penalties if the refusal is based on the inspector's lack of a warrant and one of the exemptions discussed in Part C does not apply. If the owner were to allow the inspector to enter his establishment only in response to a threat of enforcement liability, it is quite possible that any evidence obtained in such an inspection would be inadmissible. An inspector may, however, inform the owner who refuses entry that he intends to seek a warrant to compel the inspection. In any event, when entry is

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[1] FIFRA inspections are arguably not subject to this aspect of Barlow's See discussion, p. 5 and 6.

refused, the inspector should leave the premises immediately and telephone the designated Regional Enforcement Attorney as soon as possible for further instructions. The Regional Enforcement Attorney should contact the U.S. Attorney's Office for the district in which the establishment desired to be inspected is located and explain to the appropriate Assistant United States Attorney the need for a warrant to conduct the particular inspection. The Regional Attorney should arrange for the United States Attorney to meet with the inspector as soon as possible. The inspector should bring a copy of the appropriate draft warrant and affidavits. Samples are provided in the appendix to this document.

(4) Headquarters Notification

It is essential that the Regions keep Headquarters informed of all refusals to allow entry. The Regional Attorney should inform the appropriate Headquarters Enforcement Attorney of any refusals to enter and should send a copy of all papers filed to Headquarters. It is necessary for Headquarters to monitor refusals and Regional success in obtaining warrants to evaluate the need for improved procedures and to assess the impact of Barlow's on our compliance monitoring programs.

C. Areas Where a Right of Warrantless Entry Still Exists

1. Emergency Situations.

In an emergency, where there is no time to get a warrant, a warrantless inspection is permissible. In Camara v. Municipal Court, 387 U.S. 523 (1967), the Supreme Court states that "nothing we say today is intended to foreclose prompt inspections, even without a warrant, that the law has traditionally upheld in emergency situations". Nothing stated in Barlow's indicates any intention by the court to retreat from this position. The Regions will always have to exercise considerable judgment concerning whether to secure a warrant when dealing with an emergency situation. However, if entry is refused during an emergency, the Agency would need the assistance of the U.S. Marshal to gain entry, and a warrant could probably be obtained during the time necessary to secure that Marshal's assistance.

An emergency situation would include potential imminent hazard situations, as well as, situations where there is potential for destruction of evidence or where evidence of a suspected violation may disappear during the time that a warrant is being obtained.

(2) FIFRA Inspections.

There are some grounds for interpreting Barlow's as not being applicable to FIFRA inspections. The Barlow's restrictions do not apply to areas that have been subject to a long standing and pervasive history of government regulation. An Agency administrative law judge held recently that even after the Barlow's decision, refusal to allow a warrantless inspection of a FIFRA regulated establishment properly subjected the owner to civil penalty. N. Jonas & Co., Inc., I.F. & R Docket No. III-121C (July 27, 1978). For the present, however, FIFRA inspections should be conducted under the same requirements applicable to other enforcement programs.

(3) "Open Fields" and "In Plain View" situations.

Observation by inspectors of things that are in plain view, (i.e., of things that a member of the public could be in a position to observe) does not require a warrant. Thus, an inspector's observations from the public area of a plant or even from certain private property not closed to the public are admissible. Observations made even before presentation of credentials while on private property which is not normally closed to the public are admissible.

D. Securing a Warrant

There are several general rules for securing warrants. Three documents have to be drafted: (a) an application for a warrant, (b) an accompanying affidavit, and (c) the warrant itself. Each document should be captioned with the District Court of jurisdiction, the title of the action, and the title of the particular document.

The application for a warrant should generally identify the statutes and regulations under which the Agency is seeking the warrant, and should clearly identify the site or establishment desired to be inspected (including, if possible, the owner and/or operator of the site). The application can be a one or two page document if all of the factual background for seeking the warrant is stated in the affidavit, and the application so states. The application should be signed by the U.S. Attorney or by his Assistant U.S. Attorney.

The affidavits in support of the warrant application are crucial documents. Each affidavit should consist of consecutively numbered paragraphs, which describe all of the facts that support warrant issuance. If the warrant is sought in the absence of probable cause, it should recite or incorporate the neutral administrative scheme which is the basis for inspecting the particular establishment. Each affidavit should be signed by someone with personal knowlege of all the facts stated. In cases where entry has been denied, this person would most likely be the inspector who was denied entry. Note that an affidavit is a sworn statement that must either by notarized or personally sworn to before the magistrate.

The warrant is a direction to an appropriate official (an EPA inspector, U.S. Marshal or other Federal officer) to enter a specifically described location and perform specifically described inspection functions. Since the inspection is limited by the terms of the warrant, it is important to specify to the broadest extent possible the areas that are intended to be inspected, any records to be inspected, any samples to be taken, any articles to be seized, etc. While a broad warrant may be permissible in civil administrative inspections, a vague or overly broad warrant will probably not be signed by the magistrate and may prove susceptible to constitutional challenge The draft warrant should be ready for the magistrate's signature at the time of submission via a motion to quash and suppress evidence in Federal District court. Once the magistrate signs the draft warrant, it is an enforceable document. Either following the magistrate's signature or on a separate page, the draft warrant should contain a "return of service" or "certificate of service". This portion of the warrant should indicate upon whom the warrant was personally served and should be signed and dated by the inspector. As they are developed, more specific warrant-issuance documents will be drafted and submitted to the Regions.

E. Standards or Bases for the Issuance of Administrative Warrants.

The Barlow's decision establishes three standards or bases for the issuance of administrative warrants. Accordingly, warrants may be obtained upon a showing: 1) of traditional criminal probable cause, 2) of civil probable cause, or 3) that the establishment was selected for inspection pursuant to a neutral administrative inspection scheme.

1. Civil specific probable cause warrant.

Where there is some specific probable cause for issuance of a warrant such as an employee complaint or competitor's tip, the inspector should be prepared to describe to the U.S. Attorney in detail the basis for this probable cause.

The basis for probable cause will be stated in the affidavit in support of the warrant. This warrant should be used when the suspected violation is one that would result in a civil penalty or other civil action.

2. Civil probable cause based on a neutral administrative inspection scheme.

Where there is no specific reason to think that a violation has been committed, a warrant may still be issued if the Agency can show that the establishment is being inspected pursuant to a neutral administrative scheme. As the Supreme Court stated in Barlow's:

> "Probable cause in the criminal law sense is not required. For purposes of an administrative search, such as this, probable cause justifying the issuance of a warrant may be based not only on specific evidence of an existing violation, but also on a showing that "reasonable legislative or administrative standards for conducting an . . . inspection are satisfied with respect to a particular [establishment]". A warrant showing that a specific business has been chosen for an OSHA search on the basis of a general administrative plan for the enforcement of the act derived from neutral sources such as, for example, dispersion of employees in various type of industries across a given area, and the desired frequency of searches in any of the lesser divisions of the area, would protect an employers Fourth Amendment rights."

Every program enforced by the Agency has such a scheme by which it prioritizes and schedules its inspections. For example, a scheme under which every permit holder in a given program is inspected on an annual basis is a satisfactory neutral administrative scheme. Also, a scheme in which one out of every three known PCB transformer repair shops is inspected on an annual basis is satisfactory, as long as, neutral criteria such as random selection are used to select the individual establishment to be inspected. Headquarters will prepare and transmit to the Regions the particular neutral administrative scheme under which each program's inspections are to be conducted. Inspections not based on specific probable cause must be based on neutral administrative schemes for a warrant to be issued. Examples of two neutral administrative schemes are provided in the appendix. (Attachments II and III)

The Assistant U.S. Attorney will request the inspector to prepare and sign an affidavit that states the facts as he knows them. The statement should include the sequence of events culminating in the refusal to allow entry and a recitation of either the specific probable cause or the neutral administrative scheme which led to the particular establishment's selection for inspection. The Assistant U.S. Attorney will then present a request for an inspection warrant, a suggested warrant, and the inspector's affidavit to a magistrate or Federal district court judge.[2]

3. Criminal Warrants.

Where the purpose of the inspection is to gather evidence for a criminal prosecution, the inspector and the Regional Attorney should request that the U.S. Attorney seek a criminal warrant under Rule 41 of the Federal Rules of Criminal Procedure. This requires a specific showing of probable cause to believe that evidence of a crime will be discovered. Agency policy on the seeking of criminal warrants has not been affected by Barlow's. The

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[2] The Barlow's decision states that imposing the warrant requirement on OSHA would not invalidate warrantless search provisions in other regulatory statutes since many such statutes already "envision resort

distinction between administrative inspections and criminal warrant situations is discussed in Section II.A.2.

F. Inspecting with a Warrant

Once the warrant has been issued by the magistrate or judge, the inspector may proceed to the establishment to commence or continue the inspection. Where there is a high probability that entry will be refused even with a warrant or where there are threats of violence, the inspector should be accompanied by a U.S. Marshal when he goes to serve the warrant on the recalcitrant owner. The inspector should never himself attempt to make any forceful entry of the establishment. If the owner refuses entry to an inspector holding a warrant but not accompanied by a U.S. Marshal, the inspector should leave the establishment and inform the Assistant U.S. Attorney and the designated Regional Attorney. They will take appropriate action such as seeking a citation for contempt. Where the inspector is accompanied by a U.S. Marshal, the Marshal is principally charged with executing the warrant. Thus, if a refusal or threat to refuse occurs, the inspector should abide by the U.S. Marshal's decision whether it is to leave, to seek forcible entry, or otherwise.

The inspector should conduct the inspection strictly in accordance with the warrant. If sampling is authorized, the inspector must be sure to carefully follow all procedures, including the presentation of receipts for all samples taken. If records or other property are authorized to be taken, the inspector must receipt the property taken and maintain an inventory of anything taken from the premises. This inventory will be examined by the magistrate to assure that the warrant's authority has not been exceeded.

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2 continued from page 8.

to Federal court enforcement when entry is refused". There is thus some question as to whether the existence of a non-warrant Federal court enforcement mechanism in a statute requires the use of that mechanism rather than warrant issuance. We believe that the *Barlow's* decision gives the agency the choice of whether to proceed through warrant issuance or through an application for an injunction, since the decision is largely based on the fact that a warrant procedure imposes virtually no burden on the inspecting agency. In addition, an agency could attempt to secure a warrant prior to inspection on an *ex parte* basis, something not available under normal injunction proceedings. Several of the acts enforced by EPA have provisions allowing the Administrator to seek injunctive relief to assure compliance with the various parts of a particular statute. There may be instances where it would be more appropriate to seek injunctive relief to gain entry to a facility than to attempt to secure a warrant for inspection, although at this point we cannot think of any. However, since the warrant process will be far more expeditious than the seeking of an injunction, any decision to seek such an injunction for inspection purposes should be cleared through appropriate Headquarters staff.

G. Returning the Warrant.

After the inspection has been completed, the warrant must be returned to the magistrate. Whoever executes the warrant, (i.e., whoever performs the inspection), must sign the return of service form indicating to whom the warrant was served and the date of service. He should then return the executed warrant to the U.S. Attorney who will formally return it to the issuing magistrate or judge. If anything has been physically taken from the premises, such as records or samples, an inventory of such items must be submitted to the court, and the inspector must be present to certify that the inventory is accurate and complete.

III. Conclusion

Except for requiring the Agency to formalize its neutral inspection schemes, and for generally ending the Agency's authority for initiating civil and/or criminal actions for refusal to allow warrantless inspections, Barlow's should not interfere with EPA enforcement inspections.

Where there is doubt as to how to proceed in any entry case, do not hesitate to call the respective Headquarters program contact for assistance.

Marvin B. Durning

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